AF/IFW

Atty. Dkt. No. 029318-0978

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicants:	H. William BOSCH et al.
Title:	NOVEL GLIPIZIDE COMPOSITIONS
Appl. No.:	10/701,064
Filing Date:	11/05/2003
Examiner:	Susan T. TRAN
Art Unit:	1615
Confirmation No.	6295

BRIEF ON APPEAL

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Sir:

Under the provisions of 37 C.F.R. § 41.37, this Appeal Brief is being filed together with a credit card payment form in the amount of $510 covering the 37 C.F.R. 41.20(b)(2) fee for filing a brief in support of an appeal. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741. Appellants hereby appeal the Advisory Action rejection of claims 1-15, 17-24, 36-75 and 87-90 in the above-identified application to the Board of Appeals and Interferences.

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REAL PARTY IN INTEREST

The real party of interest is Elan Pharma International Limited.

RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeal or interferences.

STATUS OF CLAIMS

Claims 25-35 and 76-86 are cancelled and claims 1-24, 36-75 and 87-90 are pending in the application, with claims 1, 16, 40 and 58 being the independent claims. Claim 16 is allowed. Claims 1-15, 17-24, 36-75 and 87-90 are rejected and appealed. A copy of the pending claims is presented in the APPENDIX of this Brief.

STATUS OF AMENDMENTS

An amendment after the Final Rejection dated April 6, 2007, was filed on July 6, 2007, to comply with the Examiner's request to recite the percentage amount ranges of the glipizide and surface stabilizer in independent claims 1, 40 and 58. Claims 7-8, 49-50 and 64-65 were also amended to avoid redundancy. Further, the amendment after final amended claim 16 to be an independent claim, as requested by the Examiner, and cancelled claims 25-35 and 76-86. All amendments have been entered.

SUMMARY OF CLAIMED SUBJECT MATTER

The subject matter as claimed in each independent claim is presented below. The citation to the specification follows the procedure used in the Board's Standing Order for Patent Interferences, Paragraph 110.

Independent claim 1 reads as follows:

1. A composition comprising:

(a) particles of glipizide or a salt thereof {**page 22, lines 25-28**} having an effective average particle size of less than about 2000 nm {**page 29, lines 1-2 and lines 12-14**}; and

(b) at least one surface stabilizer {**page 6, lines 14-16**};

wherein the glipizide or a salt thereof is present in an amount of from about 99.5% to about 0.001%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients {**page 30. lines 6-9**}; and

wherein the at least one surface stabilizer is present in an amount of from about 0.5% to about 99.999% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients {**page 30, lines 10-13**}.

Claim 16 reads as follows:

16. A composition comprising:

(a) particles of glipizide or a salt thereof {**page 22, lines 25-28**}, wherein the glipizide particles have an effective average particle size of less than about 2000 nm {**page 29, lines 1-2 and lines 12-14**};

(b) at least one surface stabilizer {**page 6, lines 14-16**}; and

(c) at least one additional glipizide composition having an effective average particle size which is different from the effective average particle size of the glipizide particles of (a) {**page 19, lines 4-22**}.

Claim 40 reads as follows:

40. A method of making a glipizide composition comprising contacting particles of glipizide or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a glipizide composition having an effective average particle size of less than about 2000 nm {**page 6, lines 23-25; page 29, lines 1-2 and lines 12-14**}

wherein the glipizide or a salt thereof is present in an amount of from about 99.5% to about 0.001%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients {**page 30. lines 6-9**}; and

wherein the at least one surface stabilizer is present in an amount of from about 0.5% to about 99.999% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients {**page 30, lines 10-13**}.

Claim 58 reads as follows:

58. A method of treating diabetes in a subject in need thereof {**page 6, lines 28-31**} comprising administering to the subject an effective amount of a composition {**page 32, lines 17-30**} comprising:

(a) particles of a glipizide or a salt thereof, wherein the glipizide particles have an effective average particle size of less than about 2000 nm { **page 32, lines 17-30; page 29, lines 1-2 and lines 12-14**}; and

(b) at least one surface stabilizer {**page 6, lines 14-16**},

wherein the glipizide or a salt thereof is present in an amount of from about 99.5% to about 0.001%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients {**page 30. lines 6-9**};

wherein the at least one surface stabilizer is present in an amount of from about 0.5% to about 99.999% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients {**page 30, lines 10-13**}.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Appellants present two grounds of rejection for consideration on appeal.[1]

Specifically, Appellants present for consideration the rejection of claims 1-8, 10-11, 13-15, 17-35, 40-43, 45-50, 52-53, 55-65, 67-68 and 70-90 under 35 U.S.C. § 103(a) as being allegedly unpatentable over U.S. Patent No. 5,145,684 to Liversidge *et al.* ("Liversidge") in view of U.S. Patent No. 5,024,843 to Kuczynski *et al.* ("Kuczynski").

Further, Appellants present for consideration the rejection of claims 9, 12, 44, 51, 54, 66 and 69 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Liversidge in view of Kuczynski and international application WO 98/07414 to Parikh et al. ("Parikh").

[1] The Final Office Action dated April 6, 2007, also sets forth a Written Description rejection and an Enablement rejection of claims 25-35 and 76-86, and two provisional nonstatutory obviousness-type double patenting rejections of claims 1-15 and 17-57. Applicants canceled claims 25-35 and 76-86 in the Amendment and Reply filed on July 6, 2007, and submitted therewith Terminal Disclaimers. Accordingly, these rejections are moot.

GROUPING OF CLAIMS

Claims 1-15, 17-24, 36-75 and 87-90 stand and fall together.

SUMMARY OF THE ARGUMENTS

Claim 1 recites a composition comprising *glipizide particles having an effective average particle size of less than about 2000 nm.* Claim 40 recites a method of providing a *glipizide particle composition having an effective average particle size of less than about 2000 nm.* Claim 58 recites a method of treating diabetes in a subject comprising administering to the subject a composition comprising *glipizide particles having an effective average particle size of less than about 2000 nm.*

During prosecution, the Examiner alleged that Liversidge teaches particles of a crystalline drug and a surface modifier having an effective average particle size of less than 400 nm. Liversidge discloses anti-diabetic agents in the laundry list of drug substances that may be suitable for the invention. Applicants assert that Liversidge fails to suggest glipizide particles having an effective average particle size of less than about 2000 nm.

The Examiner recognized the deficiencies of Liversidge in failing to disclose glipizide. The Examiner relied on the disclosure of Kuczynski for the teachings that glipizide is an antidiabetic drug. The Examiner concluded that it would have been obvious to select glipizide as an anti-diabetic drug and formulate it into nanoparticles as claimed. Kuczynski, however, does not teach nanoparticulate glipizide compositions.

In essence, the Examiner applies an incorrect legal standard by persistently asserting that the disclosure of anti-diabetic agents in Liversidge renders obvious the use of any known anti-diabetic agent. To the contrary, the M.P.E.P. "Genus-Species Guidelines"[2] state that "the fact that a claimed species or subgenus is encompassed by a prior art genus is not sufficient by itself to establish a *prima facie* case of obviousness." The Examiner has not provided any compelling evidence to show *prima facie* obviousness in selecting glipizide among the vast number of anti-

[2] M.P.E.P., section 2144.08. *See* also *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994) and *In re Deuel*, 51 F.3d 1552, 1559, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995).

diabetic agents encompassed by the anti-diabetic agents genus disclosed by Liversidge, thereby to arrive at the claimed nanoparticulate glipizide composition.

Nevertheless, in the Advisory Action of July 23, 2007, the Examiner alleged that it would have been obvious to one of ordinary skill in the art to modify the nanoparticles of Liversidge using glipizide as an anti-diabetic agent, because Liversidge discloses anti-diabetic agents and Kuczynski teaches that glipizide is a well known anti-diabetic agent.

ARGUMENTS

A. The Rejection of Claims 1-8, 10-11, 13-15, 17-35, 40-43, 45-50, 52-53, 55-65, 67-68 and 70-90 under 35 U.S.C. § 103(a) over Liversidge in view of Kuczynski

The Supreme Court recently reaffirmed the Graham factors for determining obviousness.[3] The Graham factors, as outlined by the Supreme Court[4], are: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the claimed invention and the prior art; 3) resolving the level of ordinary skill in the pertinent art; and 4) evaluating evidence of secondary consideration. The Supreme Court recognized that a showing of "teaching, suggestion, or motivation" to combine the prior art to meet the claimed subject matter could provide a helpful insight in determining whether the claimed subject matter is obvious under 35 U.S.C. § 103(a), and held that the proper inquiry for determining obviousness is whether the improvement is more than the predictable use of prior art elements according to their established functions. The Court noted that it is "*important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the [prior art] elements" in the manner claimed.*

Appellants' claims are patentable over the combination of Liversidge and Kuczynski because there is no reason that would have prompted a person of ordinary skill in the relevant field to combine the elements now claimed in Appellants' invention. Appellants assert there is no reason to combine the element in the claimed fashion because (I) there is no predictive principle that all drugs may be formulated into nanoparticles; (II) the fact that a claimed species is encompassed by a genus disclosed in the prior art is not sufficient by itself to establish a *prima facie* case for obviousness; (III) the genus of anti-diabetic agents is enormous and each class of anti-diabetic agents has different properties and different mechanisms of action; (IV) *In re Spada*

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[3] *KSR Int'l Co. v. Teleflex Inc.* (No. 04-1350) (U.S., April 30, 2007).

[4] *Graham et al. v. John Deere Co. of Kansas City et al.*, 383 U.S. 1 (1966).

cannot be applied because the class of compounds known as anti-diabetic agents do not possess identical compositions, and (V) the "obvious to try" standard may not be applied in the present application.

I. There is No Predictive Principle that All Drugs May Be Formulated into Nanoparticles

The art of formulating drugs into nanoparticles is highly unpredictable. The Examiner's conclusion of obviousness relies upon the inference that formulating nanoparticles of anti-diabetic agents is routine. The Examiner has, however, failed to prove that there is a predictive principle that all anti-diabetic drugs may be formulated into nanoparticles. To the contrary, there is evidence in the cited prior art that formulating drugs into nanoparticles, not just anti-diabetic agents, is highly unpredictable. Comparative examples A-F in Liversidge clearly demonstrate that not all combinations of drugs and surface stabilizers produce nanoparticulates. Rather, some of the drugs flocculate or aggregate into large particles.

Thus informed, the artisan skilled in the art would not have known *a priori* whether glipizide can be formulated into nanoparticles, particularly given the comparative examples in Liversidge. Since the prior art fails to teach that glipizide can be formulated into nanoparticles, the reasonably creative person skilled in the art would have had no ground to create from and would not have found it obvious to prepare glipizide nanoparticles. At least for this reason, the Examiner's rejection should be reversed in whole.

II. The Fact That a Claimed Species is Encompassed by a Genus Disclosed in the Prior Art is Not Sufficient by Itself to Establish a Prima Facie Case of Obviousness

The Federal Circuit has clarified the law regarding a *prima facie* case of obviousness in a genus-species situation. Specifically, the court held that "*The fact that a claimed species or subgenus is encompassed by a prior art genus is not sufficient by itself to establish a prima facie case of obviousness*".[2]

The M.P.E.P[5] sets forth the following criteria in order to determine whether there is a *prima facie* case of obviousness when a prior art reference discloses a genus: (a) the structure of the disclosed prior art genus and that of any expressly described species or subgenus within the genus; (b) any physical or chemical properties and utilities disclosed for the genus, as well as any suggested limitations on the usefulness of the genus, and any problems alleged to be addressed by the genus; (c) the predictability of the technology; and (d) the number of species encompassed by the genus taking into consideration all of the variables possible.

(a) Structural Differences

"Some teaching of a structural similarity will be necessary to suggest selection of the claimed species or subgenus". As indicated above, since anti-diabetic agents are very different in their chemical structure and physical properties, one of ordinary skill in the art would have not been motivated, in view of the disclosure of Liversidge, to select glipizide among the myriad of available anti-diabetic agents.

(b) Different Properties

As demonstrated above, each class of anti-diabetic agents has different properties. Therefore, the artisan of ordinary skill in the art would have no reason to favor one particular class of anti-diabetic agents over another or one particular anti-diabetic agent over another.

(c) Unpredictable Technology

As stated above, there is no predictive principle that all drugs may be formulated into nanoparticles, and the Examiner has failed to prove that the art of making nanoparticles with any drug is a predictable technology. To the contrary, the reference cited by the Examiner against Appellants' invention, Liversidge, clearly demonstrates that not all combinations of drugs and surface stabilizers produce nanoparticulates after milling. Rather, some of the drugs flocculate or aggregate into large particles (*see* Comparative Examples A-F).

[5] M.P.E.P., section 2144.08A

Furthermore, because of the large variability in chemical structure and physical properties of anti-diabetic agents, the artisan skilled in the art would not have known *a priori* whether every anti-diabetic agent can be formulated into nanoparticles. Thus, the artisan skilled in the art would not have found it obvious to prepare glipizide nanoparticles. These factors clearly weigh against a conclusion of a *prima facie* case of obviousness.

(d) The Immensity of the Genus

As demonstrated above, anti-diabetic agents are an immense group of drugs and chemical compounds that differ in their chemical structure, physical properties, therapeutic effects and adverse reactions. Anti-diabetic agents do not constitute a small recognizable class of compounds with common properties. Thus, the mere disclosure of the genus of anti-diabetic agents in the primary reference does not render obvious each and every anti-diabetic agent.

III. The Genus of Anti-Diabetic Agents is Enormous and Each Class of Anti-Diabetic Agents Has Different Properties and Different Mechanisms of Action

The Board is urged to consider the totality of the record to the extent sufficient to satisfy itself. Appellants surveyed the anti-diabetic agents' literature and identified a great number of publications that amply illustrate the state of the art before Appellants' invention. The references listed below clearly show that an overwhelming number of anti-diabetic agents was available before Appellants' invention and demonstrates that each class of anti-diabetic agents has a different mechanism of action and relative advantages and disadvantages:

Koski, RR "Practical Review of Oral Antihyperglycemic Agents for Type 2 Diabetes Mellitus" *American Association of Diabetes Educators*, Published by SAGE Publications (2006), attached as Exhibit A.

Wagman, AS and Nuss, JM "Current Therapies and Emerging Targets for the Treatment of Diabetes" *Current Pharmaceutical Design 7*: 417-50 (2001), attached as Exhibit B.

Inzucchi, SE "Oral Antihyperglycemic Therapy for Type 2 Diabetes" *JAMA 287 (3)*: 360-372 (2002), attached as Exhibit C.

DeWitt, DE and Hirsch, IB "Outpatient Insulin Therapy in Type 1 and Type 2 Diabetes Mellitus", *JAMA 289 (17)*: 2254-2264 (2003), attached as Exhibit D.

Zhou, YP and Grill, VE "Long-Term Exposure of Rat Pancreatic Islets to Fatty Acids Inhibits Glucose-Induced Insulin Secretion and Biosynthesis through a Glucose Fatty Acid Cycle" *J. Clin. Invest. 93*: 870-876 (1994), attached as Exhibit E.

U.S. Patent No. 5,972,881, attached as Exhibit F.

U.S. Patent No. 5,998,463, attached as Exhibit G.

The genus of anti-diabetic agents includes, among others, biguanides, such as metformin and phenformin; glucosidase inhibitors, such as precose, acarbose, miglitol, emiglitate, voglibose and camiglibose; insulins, insulin analogues and insulin secretagogues; meglitinides, such as nateglinide, repaglinide and mitiglinide; sulfonylureas, such as acetohexamide, chlorpropamide, gliclazide, glibenclamide, glimepiride, glipizide, glyburide, tolazamide and tolbutamide; biguanide/glyburide combinations, such as Glucovance®™; thiazolidinediones, such as troglitazone, rosiglitazone and pioglitazone; PPAR-alpha agonists; PPAR alpha/gamma dual agonists; glycogen phosphorylase inhibitors; RXR agonists; imidazolines; fatty acid oxidation inhibitors; inhibitors of fatty acid binding protein (aP2); and SGLT2 inhibitors.

People affected by Type 2 diabetes Mellitus have insulin resistance, impaired insulin secretion and/or increased hepatic glucose production. Diet, exercise, anti-diabetic medications and insulin are the available therapies for the treatment of patients suffering from Type 2 Diabetes Mellitus. Anti-diabetic agents are used in people who fail to meet the glycemic goals with diet and exercise. Insulin and insulin analogues are used when oral therapy fails or is not well tolerated by the patient. Since each class of anti-diabetic agents has its unique mechanism

of action, adverse effects and prescribing precautions, pharmacologic therapy is tailored to the goals and needs of each individual patient. Thus, when selecting an anti-diabetic agent for treatment, the effects on glucose and lipids, adverse reaction profile and route of elimination must be considered prior to administration to the patient.

Following is a description of few of the many classes of anti-diabetic agents that were available before Appellants' invention. Each class includes compounds that differ in their chemical structure and physical properties, such that each class of anti-diabetic agents has its unique mechanism of action and relative advantages and disadvantages.

a) Metformin is the only biguanide FDA-approved for use in the United States. Metformin is an insulin sensitizer, as it acts by decreasing hepatic glucose production and glucose absorption in the presence of insulin, and by increasing glucose uptake into skeletal muscle. Metformin is a very attractive option for patients, as it decreases triglyceride concentrations, LDL cholesterol, total cholesterol and body weight, and increases HDL cholesterol. Additionally, metformin does not affect insulin secretion, so it does not cause hypoglycemia. Metformin, however, should be used with caution in the elderly, in patients with congestive heart failure and in patients with acute conditions predisposing them to acute renal failure or acidosis. Additionally, metformin is contraindicated in 1) patients with high level of serum creatinine; 2) patients with hepatic dysfunction; 3) patients with congestive heart failure requiring pharmacologic treatment; 4) patients with a history of drinking; and 5) patients with acute or chronic lactic acidosis. Side effects, especially gastrointestinal disturbances, and asymptomatic subnormal B12 levels, are common.

b) Alpha-glucosidase inhibitors include acarbose, miglitol, emiglitate, voglibose, MDL-25,637, camiglibose and MDL-73,945. Acarbose (Precose®) and miglitol (Glyset®) are FDA-approved for use in the United States. Alpha-glucosidase inhibitors block the action of alpha-glucosidase enzymes at the brush border of the intestine. The inhibition slows the breakdown of dietary oligosaccharides and disaccharides and the subsequent delayed digestion of

carbohydrates decreases post-prandial glucose concentrations. Although alpha-glucosidase inhibitors are less efficient than other drugs, these agents are attractive because they have minimal effect on cholesterol and body weight. Flatulence and gastrointestinal pain are common side effects in patients taking alpha-glucosidase inhibitors. In addition, acarbose may cause elevations in liver function tests. Miglitol is excreted primarily by the kidneys and should be used with caution in moderate to severe renal failure.

c) Insulin products include: 1) rapid-acting insulins, such as Lispro and Aspart, which are rapidly absorbed; 2) short-acting or regular insulin, which acts almost immediately when injected intravenously; 3)intermediate-acting insulin, such as neutral protamine Lispro (insulin lispro protamine, NPL), protamine crystalline aspart, neutral protamine Hagedorn (isophane insulin, NPH), which is slowly adsorbed due to the addition of protamine to regular insulin, and Lente insulin, which is regular insulin bound to zinc and has a slightly longer effective duration than NPH; 4) long-acting insulin, such as the slowly-adsorbed Ultralente insulin (insulin zinc extended) and the extended release insulin glargine. The major and most common adverse effects of insulin therapy are hypoglycemia and weight gain. Rate of absorption and onset and duration of action are the most variable factors among the different types of insulin available, and adjustments in the regimen, dosage and type or types of insulin administered to the patient are often necessary to correct hypoglycemia.

d) Insulin secretagogues include linogliride, insulinotropin, exendin-4 (Exenatide), BTS-67582 and A-4166.

e) Repaglinide (Prandin®) and nateglinide (Starlix®) are FDA-approved meglitinides for use in the United States. Because of their rapid onset, meglitinides stimulate pancreatic insulin secretion immediately after meal ingestion and thus attenuate post-prandial glucose excursions. Because of their short duration of action, the incidence of hypoglycemia associated with meglitinides is reduced. However, meglitinides need to be administered

frequently, may increase body weight and should not be administered to patients with impaired liver or kidney function, because they are hepatically metabolized and renally cleared.

f) Sulfonylureas lower blood sugar by binding to the sulfonylurea receptor on the membrane of beta cells in the pancreas. They facilitate calcium transport into the cells and increase endogenous insulin secretion. These compounds may be used only in patients with viable β-cells. Sulfonylureas have no effect on triglycerides or cholesterol and most of them undergo renal elimination. There is some evidence that sulfonylureas also sensitize β-cells to glucose, limit glucose production in the liver, decrease lipolysis and decrease release of insulin from the liver. Various sulfonylureas have different pharmacokinetics. Second generation sulfonylureas (glipizide, glyburide and glimepiride) have increased potency by weight, compared to first-generation sulfonylureas (acetohexamide, chlorpropamide, tolazamide and tolbutamide), but have essentially equal efficacy and are more expensive. Side effects of sulfonylureas include hypoglycemia, weight gain, dermatological and hematological reactions and gastrointestinal disturbances. The choice depends on the propensity of the patient to develop hypoglycemia.

g) Glucovance® is a fixed combination of glyburide and metformin. Combination therapies involving two or three drug classes having distinct mechanisms of action, such as sulfonylurea and metformin, metformin and thiazolidinediones and sulfonylurea and thiazolidinediones, improve glycemic control, decrease overall drug dosing and thus minimize side effects.

h) Thiazolidinediones include ciglitazone, troglitazone, rosiglitazone (Avandia®), pioglitazone (Actos®), englitazone and darglitazone. Thiazolidinediones act by stimulating the peroxisome proliferative-insulin-activated receptor gamma (PPAR) that increases insulin-stimulated glucose uptake in skeletal muscle cells and adipose tissue, and by inhibiting hepatic gluconeogenesis. When used as monotherapy, both rosiglitazone and pioglitazone do not cause hypoglycemia and raise HDL cholesterol. However, thiazolidinediones are associated with weight gain and may cause an increase in plasma volume that results in edema, and small

decreases in hemoglobin and hematocrit. Rosiglitazone and pioglitazone should be used with caution in patients with advanced congestive heart failure.

i) PPAR alpha agonists, which include bezafibrate, clofibrate, gemfibrozil, fenofibrate, ciprofibrate, and bezafibrate, reduce glucose-induced insulin secretion, lower plasma triglycerides and cholesterol levels, elevate the level of plasma HDL cholesterol and are beneficial in the prevention of ischemic heart disease in individuals with dyslipidemia.

j) Dual PPAR alpha/gamma agonists are compounds that exhibit both significant PPAR alpha and PPAR gamma agonism. These compounds include dihydrocinnamate and cinnamate derivatives, L-tyrosine derivatives, phenyl propanoic acid and propanoic acid derivatives, isoxazolidinedione and oxazolidinedione derivatives, thiazolidinediones, tricyclics, carboxylic acid and malonic acid derivatives, oxobenzylglycine derivatives, fibrates, quinoline derivatives, alkanoate derivatives, phenyalkyloxy phenyl derivatives, benzamide derivatives and isoprenols.

k) Angiotensin II Type I receptor (A-2) antagonists decrease vasoconstriction associated with hyperlipidemia, dyslipidemia and hyperglycemia. A-2 antagonists include abitesartan, benzyllosartan, elisartan, embusartan, enoltasosartan, fonsartan, forasartan, glycyllosartan, milfasartan, olmesartan, opomisartan, pratosartan, ripisartan, eprosartan, candesartan, irbesartan, saprisartan, tasosartan, telmisartan, valsartan, zolasartan and losartan.

l) RXR agonists, which include the compounds JTT-501, MCC-555, MX-6054, DRF2593,GI-262570, KRP-297 and LG100268, mimic or enhance the antidiabetic effects of thiazolidinedione compounds. RXR agonists are insulin sensitizers that increase insulin-stimulated glucose uptake, lower the level of triglyceride, suppress the level of insulin and increase the level of HDL cholesterol.

m) Imidazolines, such as midaglizole, isaglidole, deriglidole, idazoxan, efaroxan and fluparoxan, are potent stimulators of insulin secretion in pancreatic β-cells.

n) Fatty acid oxidation inhibitors, such as clomoxir and etomoxir; improve fasting hyperglycemia and hypertriglyceridemia.

Additional anti-diabetic agents include beta.-agonists, such as BRL 35135, BRL 37344, Ro 16-8714, ICI D7114, CL 316,243, TAK-667, AZ40140; phosphodiesterase inhibitors, such as sildenafil, L686398 and L-386,398; lipid-lowering agents, such as benfluorex and atorvastatin; anti-obesity agents, such as fenfluramine, orlistat and sibutramine; vanadate and vanadium complexes, such as Naglivan®™, and peroxovanadium complexes; amylin and amylin derivatives, such as pramlintide and AC-137; lipoxygenase inhibitors, such as masoprocal; somatostatin analogs, such as BM-23014, seglitide, octreotide; glucagon antagonists, such as BAY 276-9955; insulin signaling agonists; insulin mimetics; PTP1B inhibitors, such as L-783281, TER17411 and TER17529; gluconeogenesis inhibitors, such as GP3034; antilipolytic agents, such as nicotinic acid, acipimox and WAG 994; glucose transport stimulating agents, such as BM-130795; glucose synthase kinase inhibitors, such as lithium chloride, CT98014 and CT98023; galanin receptor agonists; MTP inhibitors; growth hormone secretagogues; NPY antagonists, such as PD-160170, BW-383, BW1229, CGP-71683A, NGD 95-1 and L-152804; anorectic agents, including 5-HT and 5-HT2C receptor antagonists and/or mimetics, such as dexfenfluramine, Prozac®™, Zoloft®™; CCK receptor agonists, such as SR-27897B; galanin receptor antagonists; MCR-4 antagonists, such as HP-228; leptin; 11-beta-hydroxysteroid dehydrogenase type-I inhibitors; urocortin mimetics; CRF antagonists and CRF binding proteins, such as RU-486 and urocortin.

It is beyond question that the genus of anti-diabetic agents is enormous, and each class of anti-diabetic agents has a different mechanism of action and relative advantages and disadvantages. Therefore, as one of ordinary skill in the art would have appreciated, there is no gripping reason to favor one particular class of anti-diabetic agents over another or one particular anti-diabetic agent over another.

Because the Examiner has never acknowledged the enormity of the genus of anti-diabetic agents, and the M.P.E.P. mandates that the size of the genus be considered as a factor in determining the obviousness of a species, reversal in whole of the obviousness rejection is warranted on this ground.

IV. In re Spada Cannot be Applied Because Anti-Diabetic Agents Are Not Products of Identical Composition

The Federal circuit's decision for *In re Spada*[6] was based on pressure sensitive adhesives that were created by using polymers of the same monomers in overlapping ratios as disclosed in the prior art. The final polymer product of the reference was different from the product disclosed in the prior art. The Court found that "the virtual identity of monomers and procedures sufficed to support a *prima facie* case of unpatentability of Spada's polymer latexes for lack of novelty." The *In re Spada* decision, however, cannot be applied in the present case, because anti-diabetic agents are **not** a small group of products having identical chemical structure and properties. Rather, as indicated above, anti-diabetic agents are an immense group of drugs and chemical compounds that differ in their chemical structure and physical properties, such that each class of anti-diabetic agents has its unique mechanism of action. Therefore, the principle that "Products of identical chemical composition can not have mutually exclusive properties" may not be invoked.

VI. The "Obvious to Try" Standard May Not be Applied in the Present Application

The Examiner's presumption that it would be obvious to select glipizide as an anti-diabetic agent in view of the disclosure of the prior art relies, at best, on the "obvious to try" standard .

[6] *In re Spada*, 911 F.2d 705, 709, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990).

"*When the compound is not specifically named, but instead it is necessary to select portions of teachings within a reference and combine them...*", "*anticipation can only be found if the classes of substituents are sufficiently limited or well delineated*"[7].

Furthermore, "*when there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp.*"[3]

The genus of anti-diabetic agents **is not** a limited group of compounds, and **does not** present a small, finite number of predictable solutions. The selection of an anti-diabetic agent for nanoparticulate formulations requires knowledge in the specific technical field of nanoparticulate technology that the selected anti-diabetic agent can be formulated into nanoparticles. It is therefore evident that the "obvious to try" standard may not be applied in the present application because there is an immense number of known anti-diabetic agents available, and since the nanoparticulate technology is so unpredictable, it would be time-consuming and cost-inefficient for the artisan skilled in the art to determine which anti-diabetic agent can be formulated into nanoparticles and which anti-diabetic agent cannot. Therefore, the person of ordinary skill in the art would have no reason to select glipizide among the myriad of available anti-diabetic agents.

VII. Conclusion

Independent claims 1, 40 and 58 recite a composition and methods comprising glipizide particles having an effective average particle size of less than about 2000 nm. The cited prior art fails to teach or suggest glipizide particles having an effective average particle size of less than about 2000 nm. Because of the lack of predictability in formulating drugs into nanoparticles and because of the multiplicity and variety in structure, properties, mechanism of action, therapeutic effects and adverse effects of the drugs, compounds and agents encompassed by the genus of anti-diabetic agents, the artisan skilled in the art would not have known *a priori* whether every anti-diabetic agent can be formulated into nanoparticles and would have had no compelling

[7] *Ex parte A*, 17 USPQ2d 1716 (Bd. Pat. App. & Inter. 1990).

reason to select glipizide. The claimed invention is therefore non-obvious over the cited prior art. For at least these reasons, it is submitted that the invention of claims 1, 40 and 58, and depending claims 2-8, 10-11, 13-15, 17-24, 41-43, 45-50, 52-53, 55-57, 59-65, 67-68, 70-75 and 87-90 is patentable.

B. The Rejection of Claims 9, 12, 44, 51, 54, 66 and 69 under 35 U.S.C. § 103(a) over Liversidge in view of Kuczynski and Parikh

Parikh in combination with Liversidge and Kuczynski does not obviate the claimed invention because Parikh remedies none of the deficiencies of Liversidge and Kuczynski. Rather, the disclosure of Parikh is directed to compositions comprising microparticles of water-insoluble drugs prepared using a combination of one or more surface modifiers with a phospholipid. Parikh fails to teach or disclose glipizide, let alone nanoparticulate glipizide compositions.

In the Advisory Action, the Examiner stated that Parikh is relied upon for teaching the use of mixtures of surface modifiers, alleging that nanoparticulate glipizide is taught in Liversidge in view of Kuczynski.

But Liversidge in view of Kuczynski do not teach nanoparticulate glipizide compositions, as clearly demonstrated above. Therefore, no permutations of teachings from Liversidge and Kuczynski, with or without those of Parikh, could have suggested the invention of appealed claims 9, 12, 44, 51, 54, 66 and 69. Accordingly, the obviousness rejection should be reversed in whole.

CONCLUSION

Appellants respectfully solicit the Honorable Board of Patent Appeals and Interferences to reverse the rejections of record and pass the application on to allowance.

Respectfully submitted,

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CLAIM APPENDIX

1. (Previously Presented) A composition comprising:

(a) particles of glipizide or a salt thereof, wherein the glipizide particles have an effective average particle size of less than about 2000 nm; and

(b) at least one surface stabilizer;

wherein the glipizide or a salt thereof is present in an amount of from about 99.5% to about 0.001%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients; and
wherein the at least one surface stabilizer is present in an amount of from about 0.5% to about 99.999% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

2. (Original) The composition of claim 1, wherein the glipizide is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

3. (Original) The composition of claim 1, wherein the effective average particle size of the glipizide particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

4. (Previously Presented) The composition of claim 1, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, ophthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

5. (Original) The composition of claim 1 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulations, fast melt formulations, lyophilized formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

6. (Original) The composition of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

7. (Previously Presented) The composition of claim 1, wherein the glipizide or a salt thereof is present in an amount selected from the group consisting of from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

8. (Previously Presented) The composition of claim 1, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

9. (Original) The composition of claim 1, comprising at least two surface stabilizers.

10. (Original) The composition of claim 1, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

11. (Original) The composition of claim 10, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl -D-glucopyranoside; n-decyl -D-maltopyranoside; n-dodecyl -D-glucopyranoside; n-dodecyl -D-maltoside; heptanoyl-N-methylglucamide; n-heptyl--D-glucopyranoside; n-heptyl -D-thioglucoside; n-hexyl -D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl -D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl--D-glucopyranoside; octyl -D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone.

12. (Original) The composition of claim 10, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

13. (Previously Presented) The composition of claim 10, wherein the surface stabilizer is selected from the group consisting of cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-

dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, tetrabutylammonium bromide, benzyl trimethylammonium bromide,

choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

14. (Original) The composition of any of claims 10, 12, or 13, wherein the composition is bioadhesive.

15. (Original) The composition of claim 1, comprising as a surface stabilizer hydroxypropyl cellulose.

16. (Previously Presented) A composition comprising:

(a) particles of glipizide or a salt thereof, wherein the glipizide particles have an effective average particle size of less than about 2000 nm;

(b) at least one surface stabilizer, and

(c) at least one additional glipizide composition having an effective average particle size which is different from the effective average particle size of the glipizide particles of (a).

17. (Original) The composition of claim 1, additionally comprising one or more non-glipizide active agents.

18. (Original) The composition of claim 17, wherein said additionally one or more non-glipizide active agents are selected from the group consisting of nutraceuticals, amino acids, proteins, peptides, nucleotides, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics,

sedatives, astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products, blood substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, parathyroid biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

19. (Original) The composition of claim 17, wherein said additionally one or more non-glipizide active agents are selected from the group consisting of acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

20. (Original) The composition of claim 1, wherein upon administration to a mammal the glipizide particles redisperse such that the particles have an effective average particle size of less than about 2 microns.

21. (Original) The composition of claim 20, wherein upon administration the composition redisperses such that the glipizide particles have an effective average particle size

selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

22. (Original) The composition of claim 1, wherein the composition redisperses in a biorelevant media such that the glipizide particles have an effective average particle size of less than about 2 microns.

23. (Original) The composition of claim 22, wherein the biorelevant media is selected from the group consisting of water, aqueous electrolyte solutions, aqueous solutions of a salt, aqueous solutions of an acid, aqueous solutions of a base, and combinations thereof.

24. (Original) The composition of claim 22, wherein the composition redisperses in a biorelevant media such that the glipizide particles have an effective average particle size selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

25.-35. (Cancelled)

36. (Original) The composition of claim 1 formulated into a liquid dosage form, wherein the dosage form has a viscosity of less than about 2000 mPa·s, measured at 20C, at a shear rate of 0.1 (1/s).

37. (Original) The composition of claim 36, having a viscosity at a shear rate of 0.1 (1/s), measured at 20C, selected from the group consisting of from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

38. (Original) The composition of claim 36, wherein the viscosity of the dosage form is selected from the group consisting of less than about 1/200, less than about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a liquid dosage form of a non-nanoparticulate composition of glipizide, at about the same concentration per ml of glipizide.

39. (Original) The composition of claim 36, wherein the viscosity of the dosage form is selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less

than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the glipizide, at about the same concentration per ml of glipizide.

40. (Previously Presented) A method of making a glipizide composition comprising contacting particles of glipizide or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a glipizide composition having an effective average particle size of less than about 2000 nm;

wherein the glipizide or a salt thereof is present in an amount of from about 99.5% to about 0.001%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients; and

wherein the at least one surface stabilizer is present in an amount of from about 0.5% to about 99.999% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

41. (Original) The method of claim 40, wherein said contacting comprises grinding.

42. (Original) The method of claim 41, wherein said grinding comprises wet grinding.

43. (Original) The method of claim 40, wherein said contacting comprises homogenizing.

44. (Previously Presented) The method of claim 40, wherein said contacting comprises:

(a) dissolving the particles of a glipizide or a salt thereof in a solvent;

(b) adding the resulting glipizide solution to a solution comprising at least one surface stabilizer; and

(c) precipitating the solubilized glipizide having at least one surface stabilizer adsorbed on the surface thereof by the addition thereto of a non-solvent.

45. (Original) The method of claim 40, wherein the glipizide or a salt thereof is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

46. (Original) The method of claim 40, wherein the effective average particle size of the glipizide particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1000 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

47. (Previously Presented) The method of claim 40, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, ophthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

48. (Original) The method of claim 40, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

49. (Previously Presented) The method of claim 40, wherein the glipizide or a salt thereof is present in an amount selected from the group consisting of from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

50. (Previously Presented) The method of claim 40, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 5.0% to about 99.9%, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

51. (Original) The method of claim 40, utilizing at least two surface stabilizers.

52. (Original) The method of claim 40, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

53. (Original) The method of claim 52, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl -D-glucopyranoside; n-decyl -D-maltopyranoside; n-dodecyl -D-glucopyranoside; n-dodecyl -D-maltoside; heptanoyl-N-methylglucamide; n-heptyl--D-glucopyranoside; n-heptyl -D-thioglucoside; n-hexyl -D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl -D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl--D-

glucopyranoside; octyl -D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

54. (Original) The method of claim 52, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

55. (Previously Presented) The method of claim 52, wherein the surface stabilizer is selected from the group consisting of cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-

didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

56. (Original) The method of any of claims 52, 54, or 55, wherein the composition is bioadhesive.

57. (Original) The method of claim 40, utilizing hydroxypropylcellulose as a surface stabilizer.

58. (Previously Presented) A method of treating diabetes in a subject in need thereof comprising administering to the subject an effective amount of a composition comprising:

(a) particles of a glipizide or a salt thereof, wherein the glipizide particles have an effective average particle size of less than about 2000 nm; and

(b) at least one surface stabilizer,

wherein the glipizide or a salt thereof is present in an amount of from about 99.5% to about 0.001%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients;
wherein the at least one surface stabilizer is present in an amount of from about 0.5% to about 99.999% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

59. (Original) The method of claim 58, wherein the glipizide or a salt thereof is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

60. (Original) The method of claim 58, wherein the effective average particle size of the glipizide particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

61. (Previously Presented) The method of claim 58, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, ophthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

62. (Original) The method of claim 58, wherein the composition is a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, controlled release formulations, fast melt formulations, lyophilized

formulations, tablets, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

63. (Original) The method of claim 58, wherein the composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

64. (Previously Presented) The method of claim 58, wherein the glipizide or a salt thereof is present in an amount selected from the group consisting of from about 95% to about 0.1% and from about 90% to about 0.5%, by weight, based on the total combined weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

65. (Previously Presented) The method of claim 58, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 5.0% to about 99.9%, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the glipizide or a salt thereof and at least one surface stabilizer, not including other excipients.

66. (Original) The method of claim 58, utilizing at least two surface stabilizers.

67. (Original) The method of claim 58, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

68. (Original) The method of claim 67, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate,

noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl -D-glucopyranoside; n-decyl -D-maltopyranoside; n-dodecyl -D-glucopyranoside; n-dodecyl -D-maltoside; heptanoyl-N-methylglucamide; n-heptyl--D-glucopyranoside; n-heptyl -D-thioglucoside; n-hexyl -D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl -D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl--D-glucopyranoside; octyl -D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

69. (Original) The method of claim 67, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

70. (Previously Presented) The method of claim 67, wherein the surface stabilizer is selected from the group consisting of benzalkonium chloride, polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, cationic lipids, sulfonium compounds, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl

dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

71. (Original) The method of any of claims 67, 69, or 70, wherein the composition is bioadhesive.

72. (Original) The method of claim 58, utilizing hydroxypropylcellulose as a surface stabilizer.

73. (Original) The method of claim 58, additionally comprising administering one or more non-glipizide active agents.

74. (Original) The method of claim 73, wherein said additionally one or more non-glipizide active agents are selected from the group consisting of nutraceuticals, amino acids, proteins, peptides, nucleotides, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products, blood substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, parathyroid biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

75. (Original) The method of claim 73, wherein said additionally one or more non-glipizide active agents are selected from the group consisting of acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam,

thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

76.-86. (Cancelled)

87. (Original) The method of claim 58, wherein the subject is a human.

88. (Original) The method of claim 58, wherein the method is used to treat indications where blood-glucose lowering drugs are typically used.

89. (Original) The method of claim 58, wherein the method is used to treat diabetes.

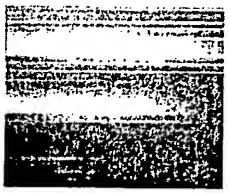
90. (Previously Presented) The method of claim 89, wherein the diabetes is non-insulin dependent diabetes mellitus.

EXHIBIT A

Practical Review of Oral Antihyperglycemic Agents for Type 2 Diabetes Mellitus

Abstract

This article gives a practical review of the pharmacology, clinical efficacy, safety, dosing, cost, and place in therapy for oral antihyperglycemic agents used in the treatment of type 2 diabetes mellitus. There are 5 classes of oral antihyperglycemic agents available in the United States: sulfonylurea secretagogues, biguanides, α-glucosidase inhibitors, thiazolidinediones, and nonsulfonylurea secretagogues. These agents have distinct characteristics that help in their selection for the treatment of type 2 diabetes.



pharmacy update

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Diet, exercise, oral antihyperglycemic medications, and insulin are the mainstay of treatment of type 2 diabetes mellitus (T2DM). Oral agents are used in people who fail to meet glycemic goals with medical nutrition therapy and exercise. Insulin is usually reserved for those who fail or cannot tolerate oral therapy. People with T2DM have insulin resistance, impaired insulin secretion, and/or increased hepatic glucose production. Oral drug treatment to date is aimed at 1 or more of these defects. The purpose of choosing the appropriate agent(s) for a person is to increase the likelihood of getting their fasting plasma glucose (FPG) and A1C to the desired glycemic goal. This article reviews the 5 oral antihyperglycemic drug classes (sulfonylurea secretagogues, biguanides, α-glucosidase inhibitors, thiazolidinediones, and nonsulfonylurea secretagogues) available in the United States with respect to mechanism of action, clinical efficacy, lipid effects, side effects, significant drug interactions, dose, cost, and place in therapy.

Sulfonylurea Secretagogues

Sulfonylureas have been available since 1954 and are classified as either first- or second-generation agents based

on when they became available. The 2 generations differ in potency, safety, and pharmacokinetics. The second-generation agents are more potent and have better pharmacokinetic and safety profiles. The first-generation agents include acetohexamide (Dymelor®), chlorpropamide (Diabinese®), tolazamide (Tolinase®), and tolbutamide (Orinase®). The second-generation agents include glimepiride (Amaryl®), glipizide (Glucotrol®), and glyburide (Diabeta®, Micronase®, Glynase®). Sulfonylureas lower FPG primarily by increasing the release of insulin from functioning pancreatic β cells. They display a glucose-dependent effect. Loss of efficacy over time is a major concern with the use of sulfonylureas. This appears to be related to exhaustion of β-cell function. Sulfonylureas increase plasma levels of insulin and cause hypoglycemia. They reduce FPG by about 60 to 70 mg/dL (3.4-3.9 mmol/L) and A1C by 1.5% to 2.0% at maximally effective doses. Sulfonylureas do not have a significant effect on lipids.[1] In the United Kingdom Prospective Diabetes Study (UKPDS), sulfonylureas were associated with a decrease in microvascular events compared to diet treatment, but there was no significant difference in mortality or macrovascular events. The lack of benefit on cardiovascular complications may be related to the fact that they cause hyperinsulinemia, which is associated with the metabolic syndrome.[2]

About 2% to 5% of patients report side effects with sulfonylureas.[1] People started on a sulfonylurea should be counseled that hypoglycemia and weight gain (typically 4 to 6 kg) are the major adverse effects.[2,3] Hypoglycemia is especially a problem with the first-generation agents because of their long half-lives. Elderly individuals, people who frequently skip meals, and people who perform frequent intense exercise are most susceptible. Given that there is a sulfonyl component in the chemical structure of sulfonylureas, hypersensitivity can occur in people with sulfa allergies and should be prescribed to people with a sulfa allergy with caution. There are drug interactions associated with sulfonylureas, including drugs that increase or decrease glucose or insulin (which would interact with all oral antihyperglycemic agents) and drugs that increase their renal excretion, increase or decrease their hepatic metabolism, or displace them from protein-binding sites.[4] It is up to the clinician to decide which are clinically significant.

Sulfonylureas should be started at low doses and titrated up every 1 to 4 weeks.[1] Dose ranges for all oral antihyperglycemic agents are listed in Table 1. Most of the effect of sulfonylureas is usually observed with half the maximum daily dose.[3] The significance of dosing oral antihyperglycemic agents up to their maximally effective dose (not their maximum daily dose) is for cost savings, avoidance of prolonged therapy that may not work, and more timely addition of another agent. Based on the results of the UKPDS, this class of drugs can be used as a first-line oral antihyperglycemic therapy, only when the person is not obese.

Biguanides

Metformin (Glucophage®) has been available since 1995 and is the only biguanide available in the United States. It is in the same drug class as phenformin, which was removed from the US and European markets in the 1970s because of its association with lactic acidosis.[1] Metformin's primary mechanism for lowering FPG is to decrease hepatic gluconeogenesis. To a smaller extent, it also improves insulin sensitivity in peripheral tissues and decreases intestinal absorption of glucose. It does not affect insulin secretion, so it does not cause hypoglycemia. Metformin's effect may diminish over time. It is unclear why this may occur. It reduces FPG by about 60 to 70 mg/dL (3.4-3.9 mmol/L) and A1C by 1.5% to 2.0%, and it is glucose dependent.[3,6] Metformin has been shown to decrease the low-density lipoprotein (LDL) levels and triglyceride levels (TGLs) by about 5 to 10 mg/dL (0.12-0.26 mmol/L) and has minimal effect on high-density lipoprotein (HDL) levels.[3,7] In the UKPDS and other more recent trials, a reduction in diabetes-related end points, deaths, and myocardial infarctions with metformin compared to diet therapy has been shown.[2,8]

About 20% to 30% of people on metformin experience gastrointestinal (GI) side effects, such as nausea, metallic taste, abdominal discomfort, and diarrhea, which can be minimized by titrating the dose up slowly and telling patients to take it with meals.[1] A modest weight loss has been associated with metformin. This weight loss is thought to result from a reduction in net caloric intake due to appetite suppression and/or a reduction in hyperinsulinemia related to insulin resistance. The reported incidence of lactic acidosis with metformin is 0.03 per 1000 patient-years of use. It is fatal in 30% to 50% of cases.[9] A recent systematic review found no cases of lactic acidosis associated with metformin use in T2DM when contraindications were followed.[10] It is contraindicated in people with the following risk factors for lactic acidosis: renal (serum creatinine ≥1.5 mg/dL [132.6 µmol/L] in men or ≥1.4 mg/dL [123.8 µmol/L] in women) or hepatic impairment,

Table 1

Doses and Costs Associated With Oral Antihyperglycemic Agents[4,5]

Drug	Dose*	Retail Price for 1 Month Supply, Low Doses (Generic)
Sulfonylureas		
Acetohexamide (Dymelor®)	250-1500 mg QD (QD-BID)	250 mg BID no data
Chlorpropamide (Diabinese®)	100-750 mg QD	250 mg QD $31.52 ($10.50)
Tolazamide (Tolnase®)	100-1000 mg QD (QD-BID)	250 mg QD $34.64 ($17.62)
Tolbutamide (Orinase®)	1000-3000 mg QD (BID-TID)	500 mg BID no data ($6.00)
Glimepiride (Amaryl®)	1-8 mg QD	2 mg QD $19.99 ($13.99)
Glipizide		
(Glucotrol®)	2.5-40 mg QD (BID)	5 mg BID $28.34 ($9.99)
(Glucotrol XL®)	5-20 mg QD	10 mg QD $29.99 ($19.99)
Glyburide		
(Diabeta®, Micronase®)	2.5-20 mg QD (BID)	2.5 mg BID $45.84 ($15.00)
Micronized glyburide (Glynase®)	0.75-12 mg QD	3 mg QD $28.60 ($12.00)
Biguanides		
Metformin		
(Glucophage®)	1000-2550 mg QD (BID-TID)	500 mg TID $70.49 ($50.98)
(Glucophage XR®)	500-2000 mg QD	1500 mg QD $69.98 ($65.98)
α-Glucosidase inhibitors		
Acarbose (Precose®)	25-100 mg with meals	25 mg TID $67.87
Miglitol (Glyset®)	25-100 mg with meals	25 mg TID $59.92
Thiazolidinediones		
Pioglitazone (Actos®)	15-45 mg QD	15 mg QD $102.55
Rosiglitazone (Avandia®)	4-8 mg QD (QD-BID)	2 mg BID $130.15
Nonsulfonylurea secretagogues		
Nateglinide (Starlix®)	60-120 mg with meals	60 or 120 mg TID $101.60
Repaglinide (Prandin®)	0.5-4 mg with meals	0.5, 1, or 2 mg TID $97.09
Combinations		
Glyburide/metformin (Glucovance®)	1.25/250 QD–10/1000 mg BID	All strengths BID $55.90
Glipizide/metformin (Metaglip®)	2.5/250–10/1000 mg BID	All strengths BID $59.99
Rosiglitazone/metformin (Avandamet®)	1/500–4/1000 mg BID	1/500 mg BID $67.85 4/1000 mg BID $227.39
Rosiglitazone/glimepiride (Avandaryl®)	4/1 QD–4/2 mg BID	4/1 mg BID no data
Pioglitazone/metformin (Actosplus Met®)	15/500 QD–15/850 mg TID	15/500 mg BID no data

QD = once daily; BID = twice daily; TID = thrice daily.
*Total daily dose is listed, but the dose interval in parentheses represents the preferred interval that the daily dose should be given.

respiratory insufficiency, severe infection, alcohol abuse, and heart failure requiring pharmacological therapy. Metformin should also be used with caution in elderly people (especially those older than 80 years) with reduced lean body mass as their low serum creatinine would fail to detect a decrease in the glomerular filtration rate. It is recommended to monitor renal function upon initiating metformin and at least annually thereafter.[11] Metformin should be withheld immediately before a person has a procedure with radiocontrast dye, as the dye increases the risk of developing renal failure and lactic acidosis while on metformin. It should also be withheld for surgery. It can be restarted immediately after surgery if the person's renal function is normal and his or her condition is stable.[1] Metformin may interfere with vitamin B12 absorption and may lower serum vitamin B12 concentrations. Anemia has been observed in 7% of people in clinical trials. It appears to be rapidly reversible with discontinuation of the drug. It is recommended to monitor hematologic parameters at baseline and then at least annually. The mechanism for this interaction is unknown.[9]

Metformin should be initiated at 500 mg orally twice daily, with the 2 largest meals of the day to minimize GI effects.[11] Metformin XR should be started at 500 mg orally once daily with the evening meal, and beneficial effects can be seen within 1 week. The dose can be titrated every 2 weeks up to 2000 mg daily.[1] Based on the UKPDS and clinical practice, metformin is considered a first-line agent and seems to be especially useful in a person who is obese or has known insulin resistance.

α-Glucosidase Inhibitors

The α-glucosidase inhibitors have been available since 1996 and include acarbose (Precose®) and miglitol (Glyset®). These drugs slow the rate of carbohydrate absorption in the small intestine, which results primarily in a reduction in postprandial plasma glucose levels. They act as competitive, reversible inhibitors of α-glucosidase (found in the small intestine) and α-amylase (found in the pancreas), which convert nonabsorbable dietary starch and sucrose into absorbable glucose and hydrolyze complex starches, respectively. These agents do not affect insulin levels, so they do not cause hypoglycemia when used alone. When α-glucosidase inhibitors are used with another antihyperglycemic agent (ie, sulfonylureas) and hypoglycemia occurs, the hypoglycemia needs to be reversed by ingesting glucose (tablets or gels), not complex carbohydrates. Complex carbohydrates would be slowly broken down by the α-glucosidase inhibitor and thus would not be effective in quickly relieving hypoglycemia. FPG is reduced about 20 to 30 mg/dL (1.1-1.7 mmol/L) and A1C by 0.7% to 1.0%, and these agents are more effective at lowering postprandial than preprandial glucose levels since they are dosed with meals and have a short onset/offset. Postprandial glucose levels are decreased by about 40 to 50 mg/dL (2.2-2.8 mmol/L), and no significant effects on lipids have been shown.[1,12]

α-Glucosidase inhibitors are associated with GI side effects, such as abdominal discomfort (11%-21%), diarrhea (28%-33%), and flatulence (41%-77%). These effects are related to the presence of undigested carbohydrates in the lower GI tract. By titrating the dose up slowly, these effects can be minimized. People with inflammatory bowel disease, intestinal obstruction, or predisposition to obstruction, cirrhosis, or renal impairment (serum creatinine >2 mg/dL [176.8 μmol/L]) should not use these agents.[13,14]

Dosing should be initiated at 25 mg orally once or twice daily with the first bite of meals and titrated up every 2 to 4 weeks to a maximum dosage of 100 mg 3 times daily. If a meal is skipped (or added), the dose for that meal should be skipped (or added).[13,14] These agents are useful for people with high postprandial glucose levels and/or irregular meal schedules.

Thiazolidinediones

Thiazolidinediones (TZDs) have been available since 1997 and include pioglitazone (Actos®) and rosiglitazone (Avandia®). TZDs act primarily to improve insulin sensitivity of muscle and adipose tissue. To a lesser extent, they decrease hepatic glucose production. TZDs are selective and potent agonists for the peroxisome proliferator-activated receptor γ (PPARγ) nuclear receptors. Activation of these receptors regulates the transcription of insulin-responsive genes involved in the control of production, transport, and use of glucose. The action of these agents requires the presence of insulin.[3] TZDs may also improve β-cell function by reducing free fatty acids.[15] They do not directly affect insulin secretion, so they are not associated with hypoglycemia. TZDs' effects are glucose dependent, and they reduce FPG by about 35 to 40 mg/dL (2.0-2.2 mmol/L) and A1C by 1% to 1.5%.[1,16] They differ in their effects on lipids. Rosiglitazone increases LDL by 13 to 18 mg/dL (0.34-0.47 mmol/L), has no effect on TGL, and

increases HDL by 2 to 3.4 mg/dL (0.05-0.09 mmol/L). Pioglitazone has a neutral effect on LDL, decreases TGL by 26 to 53 mg/dL (0.29-0.60 mmol/L), and increases HDL by 3.61 to 5.48 (0.09-0.14 mmol/L).[16] Both agents may reduce the level of small, dense LDL cholesterol, which is thought to be the most atherogenic lipoprotein component in people with diabetes and may reduce macrovascular morbidity and mortality.[17-19] There are other PPAR agonists in early developmental stages that may have improved lipid effects and less adverse effects compared to the current agents.[20]

TZDs are associated with edema and weight gain (about 2 kg). The weight gain may be due to a change in fat distribution with an increase in subcutaneous adipose fat and a decrease in visceral fat. It could also be due to an increase in plasma volume (ie, edema). The edema is thought to be due to a decrease in renal excretion of sodium and an increase in sodium and free water retention. Pedal edema occurs in 3% to 5% of people taking TZDs. The incidence is greater when they are used with other glucose-lowering agents. Because of the edema, TZDs are contraindicated in people with New York Heart Association class 3 or 4 heart failure.[21] The increase in plasma volume can also cause dose-related dilutional anemia (decreased hemoglobin of about 1 g/dL [0.62 mmol/L], hematocrit about 3.3%).[1,21] There are rare postmarketing reports of new or worsening macular edema in people taking TZDs, and in some cases, improvement was seen with discontinuation of the drug. Most of these people also reported concurrent peripheral edema.[22] TZDs can induce ovulation in women with polycystic ovary syndrome.[4] Premenopausal and perimenopausal women with polycystic ovary syndrome must be made aware of this possibility. Pioglitazone and rosiglitazone have been associated with an increase in aspartate aminotransferase and alanine aminotransferase greater than 3 times the upper limit of normal at an incidence similar to placebo (0.25%). Troglitazone (Rezulin®) was taken off the US market in 2000 because of reports of idiosyncratic hepatocellular injury.[23] Pioglitazone and rosiglitazone do not share this hepatotoxic profile; however, these agents should be used with caution in hepatic impairment. People on TZDs should have their liver enzymes monitored at the start of therapy and then annually thereafter. If a person's alanine aminotransferase level remains elevated above 3 times the upper limit of normal, the TZD agent must be discontinued.[24,25]

Pioglitazone is initiated at 15 mg orally daily and titrated up every 3 to 4 weeks to a maximum dose of 45 mg. Rosiglitazone's starting dose is 4 mg once daily or 2 mg twice daily and is titrated up at the same interval as pioglitazone to a maximum of 8 mg/d. TZDs have a slow onset of action, so the full effect may not be seen for up to 4 months.[1] People with known insulin resistance and/or a contraindication to metformin may find TZDs of benefit.

Nonsulfonylurea Secretagogues (Meglitinide Analogues)

The nonsulfonylurea secretagogues (available since 1998) include repaglinide (Prandin®) and nateglinide (Starlix®).[1] Mitiglinide is available in Japan. It is in phase 3 trials in Europe and in phase 2 trials in the United States.[26] These agents stimulate the release of insulin from functioning pancreatic β cells if glucose is present. Repaglinide and nateglinide reduce FPG by about 65 to 75 mg/dL (3.6-4.2 mmol/L) and A1C by about 1.5% to 2.0% and 0.5% to 1.5%, respectively.[1,27,28] They have a short half-life, so they stimulate insulin release for brief episodes. The quick on and off helps decrease hypoglycemia, hyperinsulinemia, weight gain, and possible β-cell exhaustion compared to sulfonylureas. Dosed prior to meals, the maximal effect on glucose occurs postprandially. Meglitinides have no effect on lipids. Both agents are metabolized by CYP 450 3A4, and nateglinide is also metabolized by 2C9.[1,4]

Repaglinide should be initiated at 0.5 mg 3 times daily orally and titrated up to a maximum daily dose of 16 mg.[29] Most of the benefit is achieved with 1 mg 3 times daily.[1] Nateglinide should be initiated at 60 mg 3 times daily orally and titrated up to a maximum daily dose of 360 mg.[30] Doses should be taken 15 to 30 minutes prior to meals. If a meal is skipped (or added), the dose for that meal should be skipped (or added).[29,30] These agents are useful for people with high postprandial glucose levels and/or irregular meal schedules.

Combination Therapy

If monotherapy does not achieve goal glycemic levels, combination therapy is used. Most of the drug classes reviewed can be combined, except sulfonylureas and nonsulfonylurea insulin secretagogues since they have the same mechanism of action. There are combination tablets that have been formulated to allow for a decreased pill burden (see Table 1). If combination oral therapy does not achieve glycemic goals, insulin should be initiated. There

are several reasons people may fail monotherapy. First, results from the UKPDS showed that sulfonylureas and metformin are efficacious for approximately 5 years; after that, people require a change or addition in their therapy. At 3 years, 50% of the people in the UKPDS achieved glycemic goals with monotherapy. At 9 years, only 25% were at goal with monotherapy. The conclusion was that because diabetes is a progressive disease, most people need multiple therapies to continue to attain glycemic goals.[31] Also, failure of monotherapy occurs because of noncompliance. This may be because of a lack of knowledge about medications, adverse effects, cost of obtaining the medications, or other reasons. In a study in 2000, 261 people with T2DM from the United Kingdom were surveyed about their knowledge of their oral antihyperglycemic agents. Findings included that (1) 62% took their medication correctly in relation to food, (2) 10% of those taking sulfonylureas knew it may cause hypoglycemia, (3) 20% of those taking metformin were aware of its GI side effects, (4) 35% recalled receiving advice about their medication, (5) 20% forgot to take their medication at least once a week, and (6) 5% omitted doses because of hypoglycemia.[32] This is an important reminder to educate people with T2DM about the proper use of oral antihyperglycemic drugs.

Oral Antihyperglycemic Drug Classes on the Horizon

There are new classes of oral drugs in early stages of development for the treatment of T2DM, including DPP-IV enzyme inhibitors and glucokinase (GK) activators.[33,34] DPP-IV is responsible for cleaving certain polypeptides. Inhibiting this enzyme results in decreased FPG and suppression of postprandial glucose excursions. On February 21, 2006, Merck & Co, Inc announced that the new drug application (NDA) for its once-daily DPP-IV enzyme inhibitor, Januvia® (sitagliptin phosphate), was accepted for standard review by the US Food and Drug Administration (FDA). Merck expects FDA action on the NDA by mid-October. Januvia® would potentially be the first in a new class of oral medications (DPP-4 inhibitors) that enhances the body's own ability to lower blood sugar when it is elevated.[35]

GK is present in pancreatic β cells and acts as a glucose sensor to establish the threshold for glucose-stimulated insulin release. GK is also present in the liver. It controls hepatic glucose metabolism and influences glucose uptake and production. Mutations of the GK gene result in several subtypes of diabetes. Drugs that activate GK would be a novel approach to the treatment of T2DM since it is involved in glucose homeostasis.[34]

Conclusion

T2DM is a chronic disease that usually requires drug therapy. All of the available oral antihyperglycemic agents are effective at lowering plasma glucose and A1C levels. Choosing the oral antihyperglycemic agent(s) that best fits the individual person and educating them on the proper use of the medication will enhance efficacy, safety, and compliance with therapy.

References

1. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. Ann Intern Med. 1999;131:281-303.
2. American Diabetes Association. Implications of the United Kingdom Prospective Diabetes Study. Diabetes Care. 2003;26 (suppl 1):S28-S32.
3. Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes. JAMA. 2002;287:360-372.
4. Wickersham RM, Novak KK, eds. Drug Facts and Comparisons. St. Louis, Mo: Wolter Kluwer; 2006.
5. Average wholesale prices [Drugstore.com Web site]. Available at: www.drugstore.com/pharmacy/drugindex/. Accessed February 14, 2006.
6. Saenz A, Fernandez-Esteban I, Mataix A, et al. Metformin monotherapy for type 2 diabetes mellitus. Cochrane Metabolic and Endocrine Disorders Group. Cochrane Database Syst Rev. 2005;(3):CD002966.
7. Wulffele MG, Kooy A, De Zeeuw D, et al. The effect of metformin on blood pressure, plasma cholesterol and triglycerides in type 2 diabetes mellitus: a systematic review. J Intern Med. 2004;256:1-14.
8. Johnson JA, Simpson SH, Toth EL, et al. Reduced cardiovascular morbidity and mortality associated with metformin use in subjects with type 2 diabetes. Diabet Med. 2005;22:497-502.
9. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med. 2002;137:25-33.
10. Salpeter SR, Greyber E, Pasternak GA, et al. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Arch Intern Med. 2003;163:2594-2602.
11. Glucophage. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.
12. Van der Laar FA, Lucassen PLBJ, Akkermans RP, et al. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Metabolic and Endocrine Disorders Group. Cochrane Database Syst Rev. 2005;(2):CD003639.
13. Precose. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.
14. Glyset. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.

15. Shimabukuro M, Zhou Y-T, Lee Y, et al. Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. J Biol Chem. 1998;273:3547-3550.
16. Chiquette E, Ramirez G, DeFronzo R. A meta-analysis comparing the effect of thiazolidinediones on cardiovascular risk factors. Arch Intern Med. 2004;164:2097-2104.
17. Ahmann AJ, Riddle MC. Current oral agents for type 2 diabetes: many options, but which one to choose when? Postgrad Med. 2002;111:32-46.
18. Dormandy JA, Charbonnel B, Eckland DJA, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomized controlled trial. Lancet. 2005;366:1279-1289.
19. Choi D, Kim SK, Choi SH, et al. Preventative effects of rosiglitazone on restenosis after coronary stent implantation in patients with type 2 diabetes. Diabetes Care. 2004;27:2654-2660.
20. Perfetti R, D'Amico E. Rational drug design and PPAR agonists. Curr Diabetes Rep. 2005;5:340-345.
21. Nesto RW, Bell D, Bonow RO, et al. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. Diabetes Care. 2004;27:256-263.
22. Glitazone-associated macular edema. Pharmacist's Letter/Prescriber's Letter. 2006;22:220205.
23. Scheen AJ. Hepatotoxicity with thiazolidinediones: is it a class effect? Drug Saf. 2001;24:873-888.
24. Avandia. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.
25. Actos. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.
26. Mitiglinide: KAD 1229, S 21403. Drugs R D. 2004;5:98-101.
27. Phillips LS, Dunning BE. Nateglinide (Starlix™): update on a new antihyperglycemic agent. Int J Clin Pract. 2003;57:535-541.
28. Dornhorst A. Insulinotropic meglitinide analogues. Lancet. 2001; 358:1709-1716.
29. Prandin. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.
30. Starlix. In: Physicians' Desk Reference [electronic version]. Greenwood Village, Colo: Thomson Micromedex; 2006.
31. Turner RC, Cull CA, Frighi V, et al. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 40). JAMA. 1999;281:2005-2012.
32. Browne DL, Avery L, Turner BC, et al. What do patients with diabetes know about their tablets? Diabet Med. 2000;17:528-531.
33. Nielsen LL. Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes. DDT. 2005;10:703-710.
34. Sarabu R, Grimsby J. Targeting glucokinase activation for the treatment of type 2 diabetes—a status review. Curr Opin Drug Discov Devel. 2005;8:631-637.
35. Diabetes in Control.Com News and Information for Medical Professionals Issue #300. FDA accepts Merck's first in class drug application for JANUVIA™ for type 2. Available at: www.diabetes@topica.email-publisher.com. Accessed February 22, 2006.

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Practical Review of Oral Antihyperglycemic Agents for Type 2 Diabetes Mellitus

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EXHIBIT B

Current Therapies and Emerging Targets for the Treatment of Diabetes

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Abstract: Concurrent with the spread of the western lifestyle, the prevalence of all types of diabetes is on the rise in the world's population. The number of diabetics is increasing by 4-5% per year with an estimated 40-45% of individual's over the age of 65 years having either type II diabetes or impaired glucose tolerance. Since the signs of diabetes are not immediately obvious, diagnosis can be preceded by an extended period of impaired glucose tolerance resulting in the prevalence of beta-cell dysfunction and macrovascular complications. In addition to increased medical vigilance, diabetes is being combated through aggressive treatment directed at lowering circulating blood glucose and inhibiting postprandial hyperglycemic spikes. Current strategies to treat diabetes include reducing insulin resistance using glitazones, supplementing insulin supplies with exogenous insulin, increasing endogenous insulin production with sulfonylureas and meglitinides, reducing hepatic glucose production through biguanides, and limiting postprandial glucose absorption with alpha-glucosidase inhibitors. In all of these areas, new generations of small molecules are being investigated which exhibit improved efficacy and safety profiles. Promising biological targets are also emerging such as (1) insulin sensitizers including protein tyrosine phosphatase-1B (PTP-1B) and glycogen synthase kinase 3 (GSK3), (2) inhibitors of gluconeogenesis like pyruvate dehydrogenase kinase (PDH) inhibitors, (3) lipolysis inhibitors, (4) fat oxidation including carnitine palmitoyltransferase (CPT) I and II inhibitors, and (5) energy expenditure by means of beta 3-adrenoceptor agonists. Also important are alternative routes of glucose disposal such as Na^+-glucose cotransporter (SGLT) inhibitors, combination therapies, and the treatment of diabetic complications (eg. retinopathy, nephropathy, and neuropathy). With may new opportunities for drug discovery, the prospects are excellent for development of innovative therapies to effectively manage diabetes and prevent its long term complications. This review highlights recent (1997-2000) advances in diabetes therapy and research with an emphasis on small molecule drug design (275 references).

INTRODUCTION

Diabetes mellitus has been recognized as a growing world-wide epidemic by many health advocacy groups including the World Health Organization (WHO) [1]. The WHO has estimated that diabetes will be one of the worlds leading causes of death and disability within the nextquarter century. The statistics are alarming; 30 million people were diagnosed with diabetes world-wide in 1985, by 1995 the number had risen to 135 million, and at the current rate there will be some 300 million by the year 2025 as predicted by the WHO [2]. Currently, there are more than 17 million type 2 diabetic patients in the US (or ~5.9% of the population), 11 million in Europe, and 6 million in Japan; which represents a potential primary therapeutic market of over $6 billion. In the US in 1997, the American Diabetes

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Association (ADA) reports that the total economic cost of diabetes was estimated to be $98 billion which includes $44 billion in direct medical and treatment costs, and $54 billion in indirect costs related to disability and mortality. The prevalence of all forms of diabetes is estimated to be 2-3% of the world's population, with the number of diabetics increasing by 4-5% per annum. It is projected that as many as 40-45% of persons aged 65 or older have either Type 2 diabetes or its precursor state, impaired glucose tolerance (IGT). This year approximately 800,000 people will be diagnosed with diabetes. Unfortunately, not all diabetics are diagnosed. According to the ADA, about a third of those with the disease are unaware that they are suffering from hyperglycemia [3].

The recent and dramatic increase in the prevalence of diabetes can be attributed to several factors. Globally, diabetes has shadowed the spread of the so-called "modern western lifestyle", and can be linked to an increasingly overweight and sedentary population. In the US, the increase is also due to the overall aging and obesity of the population; along with heightened patient education and awareness, increased diagnosis, higher compliance with monitoring guidelines, and the newly defined lower cut-off for normal fasting plasma glucose levels (126 mg/dl). Maintaining a healthy diet and an active exercise program are important components in the prevention and treatment of diabetes. While lifestyle modifications may be preventative for some susceptible individuals, once clinically diagnosed the disease is chronic, progressively debilitating, and has no cure [4]. Even while maintaining a healthy lifestyle, the majority of patients need pharmacological intervention which might consist of one or a combination of the following oral medications: sulfonylureas (glyburide, glipizide), Metformin, or thiazolidinediones (Avandia, Actos). However 30-40% of patients are not adequately controlled by these therapies and require subcutaneous insulin injections. Thus, the need for new drugs and novel methods of treatment is apparent, and the demand for new drugs will only increase in the patient-driven healthcare systems of the industrialized countries.

Diabetes can be characterized as a disease in which the body is either dysfunctional in insulin production or insulin utilization, resulting in dysregulation of glucose metabolism. There are two major forms of diabetes; Type 1 and 2. Type 1 diabetes is typically an autoimmune disease characterized by the loss of pancreatic β-cell function and an absolute deficiency of insulin. In the US, 5-10% of diabetic population suffers from Type 1 diabetes. The remainder of the diabetic population has either Type 2 diabetes or IGT which is a failure of the body to properly respond to insulin. Compared to Type 1 diabetes, the etiology of Type 2 or IGT is varied and much more complex [5]. In either treated or untreated patients, the progression of the disease is marked by hyperglycemia, but the metabolic disorder underlying diabetes also affects protein and lipid metabolism leading to serious complications, including peripheral nerve damage, kidney damage, impaired blood circulation, and damage to the retina. Diabetes is the leading cause of blindness and amputation in western populations [5].

The United Kingdom Prospective Diabetes Study (UKPDS), a long term study of Type 2 diabetics, has shown that rigorous management of blood glucose levels (measured as glycosylated hemoglobin, HbA_{1c}), and blood pressure substantially reduced the incidence of complications [6]. The current therapeutic strategies for treatment of diabetes focus on maintaining glycemic control and median HbA_{1c} levels at or below 7% by using insulin or one of several oral agents (sulfonylureas, metformin, or thiazolidinediones) when lifestyle alone is not effective. Combination therapy with one or more of these agents is now a viable option as target blood glucose levels become harder to maintain with monotherapy [6,7]. A wide variety of therapeutic approaches are now being examined for Type 2 diabetes, and can generally be classified in one of the following categories: 1) insulin or insulin mimetics; 2) agents which effect the secretion of insulin; 3) inhibitors of hepatic glucose production; 4) insulin sensitizers; and 5) agents which inhibit glucose absorption. This review will highlight some of the most promising current and

investigational research in each of these important categories.

INSULIN, INSULIN ANALOGUES OR MODIFIED INSULIN/IMPROVED DELIVERY

Insulin/Modified Insulins

Early or timely initiation of insulin therapy, either as monotherapy or in combination with other agents, can establish good glycemic control in a majority of Type 2 patients, and this mode of therapy does not adversely effect the quality of life of these patients [7,8]. Insulin is typically injected 30 to 60 minutes after a meal, but the regiments vary from country to country. A conventional treatment uses a combination of long and short acting forms of insulin injected subcutaneously (*sc*). Ideally, insulin delivery should mimic physiological conditions in which the secretion by the pancreas peaks over basal after every snack or meal, but in the US treatment tends toward a once-a-day long-acting insulin. There are many forms of insulin available, but they mainly fall into two categories; short- and long-acting. The long-acting insulins are typically crystalline or suspensions, while short-acting insulin is a solution. Short-acting analogs of insulin such as insulin lispro (Humalog), an analog of insulin which has favorable pharmacokinetics, and is useful in the control of post-prandial glucose levels, have been recently reviewed and the reader is referred to these articles [9]. A detailed analysis of the properties and pharmacology of the various types of insulin is beyond the scope of this review.

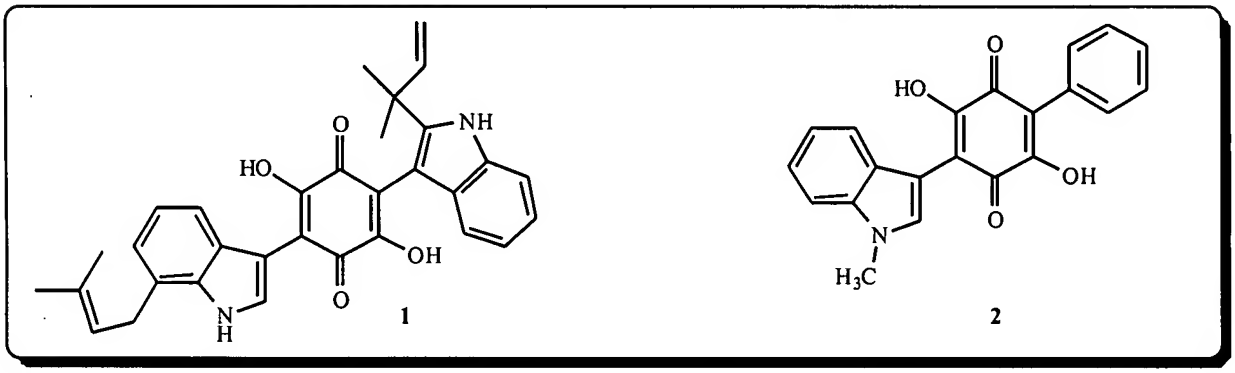
Improved Delivery Vehicles

Inhalable, oral, intranasal, and transdermal forms of insulin offer considerably more convenience than do the traditional *sc* injectable forms of the drug, along with having more favorable pharmacokinetic characteristics. The common problem associated with these alternative delivery methods is variability of dosing among the clinical population. This is partly do to patient training on these new delivery devices. Insulin pumps offer a lower chance of hypoglycemia, but also substantial health risk if they become blocked or damaged. These alternatives have also been extensively reviewed [10,11].

Insulin Mimetics

For at least the past quarter century, the quest for orally available, small molecule insulin mimetics which could replace the parenterally administered drug and diminish some of the drawbacks associated with long term insulin usage has been the "Holy Grail" of diabetes research. Despite considerable effort toward this goal, little progress has been made. Several recent reports may help to reverse this situation. Following an extensive screening campaign for molecules that activate the human insulin receptor, the natural product L-783,281 (1), a non-peptidyl fungal metabolite, was reported to selectively activate the insulin receptor *in vitro*, and oral administration of this agent showed a 40% lowering of blood glucose when administered orally for 7 days [12]. In the initial report, the proposed mechanism of action of 1 was attributed to intracellular activation of the insulin receptor tyrosine kinase (IRTK).

A recent, more detailed paper from the same group has described the ability of a series of structurally related 3,6-diaryl-2,5-dihydroxybenzoquinones to selectively activate the human insulin receptor [13]. The best of these compounds, 2,[14] a simplified derivative of 1, has been found to activate the insulin receptor tyrosine kinase with an EC_{50} of 300 nM and did not induce the activation of other, closely related tyrosine kinase activated receptors such as the insulin-like growth factor receptor (IGF1-R), epidermal growth factor receptor (EGFR), and platelet-derived growth factor (PDGFR) at concentrations up to 30 uM. Administration of 2 to hyperglycemic *db/db* mice showed a dose dependent ability to correct hyperglycemia (given at 0.1-10 mg/kg/day), and when administered to *ob/ob* mice at a dose of 10 mg/kg effected significant reduction in hyperinsulinemia. Also, it should be noted that no hypoglycemia was observed in normal mice when 2 was given at a 10 mg/kg dose. Structurally related compounds that



were inactive in the insulin receptor activation assay did not effect blood glucose levels in *db/db* mice at equivalent dosages, thus solidifying the relationship between insulin receptor activation and reduction in hyperglycemia observed with 2. Further evaluation of 2 in tests to address potential quinone-related structural liabilities, *inter alia* the uncoupling of mitochondrial respiration, catalysis of the formation of reactive oxygen species, hemolysis through heme oxidation, and the reaction of 2 with nucleophilic thiols, have shown that 2 is well behaved and has no overt toxicity. These results bode well for the future of these types of insulin "agonists" and are likely to stimulate considerable interest in this potentially important therapeutic strategy.

ENHANCERS OF INSULIN RELEASE

Sulfonylureas

Sulfonylureas (SU) remain a front line treatment for Type 2 diabetes [15]. SUs stimulate insulin release from pancreatic β-cells by a mechanism that involves blocking ATP-sensitive potassium (K+) (KATP) channels. Recently α-endosulfine, an 18-kD protein, which displaces the binding of sulfonylureas to β-cell membranes, inhibits cloned KATP channels, and stimulates insulin secretion, has been proposed as an endogenous ligand for the SU receptor [16].

Common side effects of SU therapy include hypoglycemia, as their action may occur at times when insulin is not required, and weight gain [17]. Prolonged treatment with SU's also exacerbates β-cell exhaustion through over-stimulation of insulin production; SU therapy is not effective when insulin receptor levels decline too far. Newer agents have attempted to address these liabilities. Repaglinide 3 and nateglinide 4 are recently introduced short-acting non-sulfonylureas secretagogues which are taken prior to meals to control post-prandial glucose levels and are reported to have fewer hypoglycemic side-effects [18,19]. A family of short-acting sulfonylureas, typified by 5, has recently been reported [20]. Administration of 5 at 3 mg/kg *po* lowered blood glucose levels by > 30%, 10-20%, and < 10% at 30 min, 1 hour, and 2 hours, respectively. JTT-608 6 is an insulinotropic agent which stimulates insulin release only in the presence of elevated blood glucose levels, reducing the risk of hypoglycemia [21]. In isolated rat β-pancreatic cells, 6 increases insulin secretion by enhancing Ca^{2+} efficacy and increasing Ca^{2+} influx as a result of increased intracellular cAMP levels due to inhibition of phosphodiesterase (PDE) activity.

Glucagon-like Peptide-1 (GLP-1)

The effects of glucagon-like peptide-1 (GLP-1) and analogues on insulin secretion have been recently reviewed [22]. N-terminal modified analogs of GLP-1 have been shown to increase the lifetime of this peptide [23]. Exendin-4, an GLP-1 mimetic with favorable pharmacokinetic properties, may hold promise as novel therapy to stimulate β-cell growth and differentiation [24]. Encapsulated, genetically engineered cells which can secret a mutant, long-lived form of GLP-1, and can be implanted in diabetic patients in the hopes of correcting hyperglycemia have been reported [25]. Inhibitors of dipeptidyl peptidase-IV (DPP-

IV), which is involved in the majority of GLP-inactivation *in vivo*, can also extend the lifetime of GLP-1 [26]. In a detailed biochemical study, pyrrolidine **7**, which is a potent (K_i =11 nM) and specific inhibitor of DPP-IV, has been shown to rapidly inhibit DPP-IV *in vivo* when dosed orally in rats [27]. Glucose tolerance was increased by 50% in rats receiving chronic treatment of **7**.

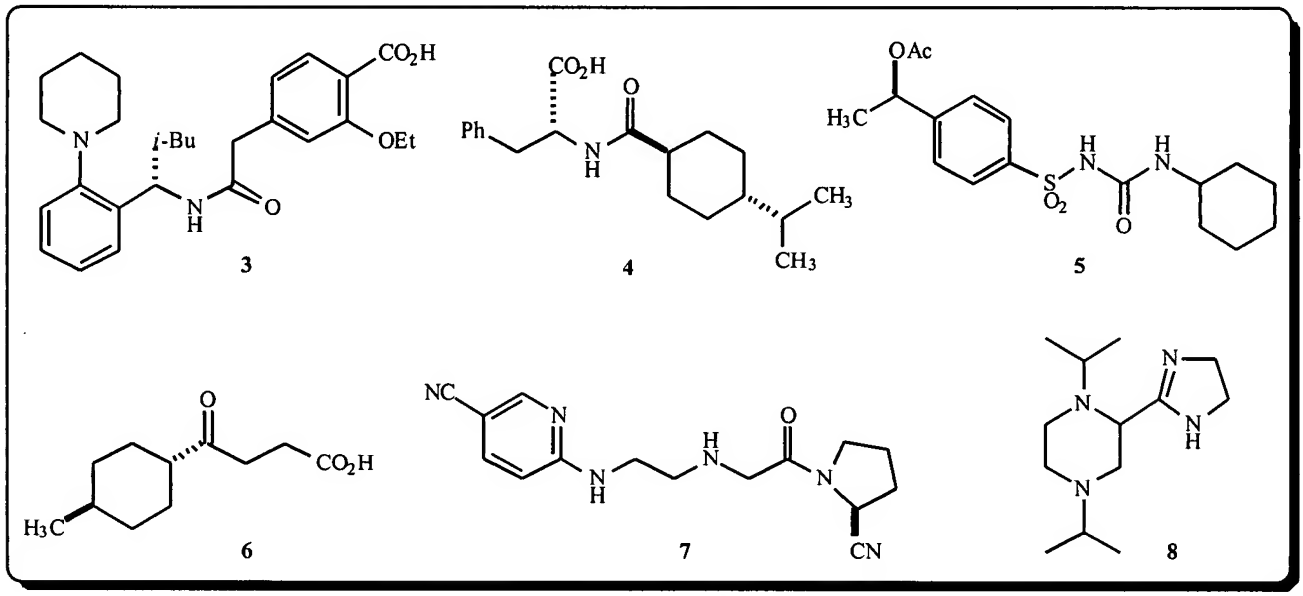
Imidazolines

S-22068 (**8**) has a potent effect on glucose tolerance and increased significantly insulin secretion in diabetic rats [28]. Unlike earlier imidazoline-based secretagogues, **8** does not have a high affinity for α-2 adrenoceptors or the known imidazoline binding sites I_1 or I_2, but may act through a novel imidazoline-binding site.

INHIBITORS OF HEPATIC GLUCOSE PRODUCTION

Consideration of Biological Regulation

Excessive hepatic glucose production (HGP) is a significant contributor to diabetic hyperglycemia and in Type 2 diabetics, HGP is significantly elevated relative to non-diabetics. Methods for the inhibition of HGP are becoming an important strategy for control of blood glucose levels [29]. Several of the novel approaches to the inhibition of HGP by effecting gluconeogenesis or glycogenolysis are discussed in this section. Drug development programs are targeting may of the regulatory enzymes involved in gluconeogenesis, lipolysis, and fatty acid oxidation. Conceptually, gluconeogenesis could be controlled by limiting the availability substrates (dichloroacetic acid) [30] or by inhibition enzymes in the pathway. At the top of the gluconeogenesis signal cascade is pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDHK). While PDHK seems to be a safe point of intervention, PC is also involved in the citric acid cycle and is expected to cause toxic side effects if inhibited. Alternatively, indirect inhibitors of PC, such as known aromatic acid acetyl-CoA sequestrants, may be useful for intervention of gluconeogenesis [31]. Excesses of nonesterified fatty acids (NEFA) have been implicated in the pathology of insulin resistance [32]. High levels of NEFA have been linked in humans with insulin insensitivity [33]. Treatment with nicotinic acid decreases NEFA and increases peripheral glucose uptake, but this effect is not long lived [34]. Perhaps a more specific analogue of nicotinic acid, such as acipimox, will give better results for controlling dyslipidemia and hyperglycemia [35]. In diabetics, increased lipolysis converts long-chain fatty acids to acetyl-CoA adducts in the liver. The long-chain fatty acid acetyl-CoA is transported to mitochondrial β-oxidation complexes by two enzymes carnitine



palmitoyltransferase I (CPT-I) and CPT-II. Early irreversible inhibitors of CPT-I were shown effective in reduction of hyperglycemia, normalizing dyslipidemia, and improving insulin sensitivity in type II diabetic patients [31]. Unfortunately, they also caused significant cardiac hypertrophy which may be related to an imbalance in metabolic energy regulation. Further study will show if mitochondrial energy metabolism is a safe target for diabetes therapy. New biological targets in liver glucose homeostasis are being discovered at a rapid pace. Reduction of HGP through inhibition of any of these pathways should be helpful in controlling diabetes and diabetic complications either as stand-alone therapies or in combination with other drugs.

Biguanides - Metformin

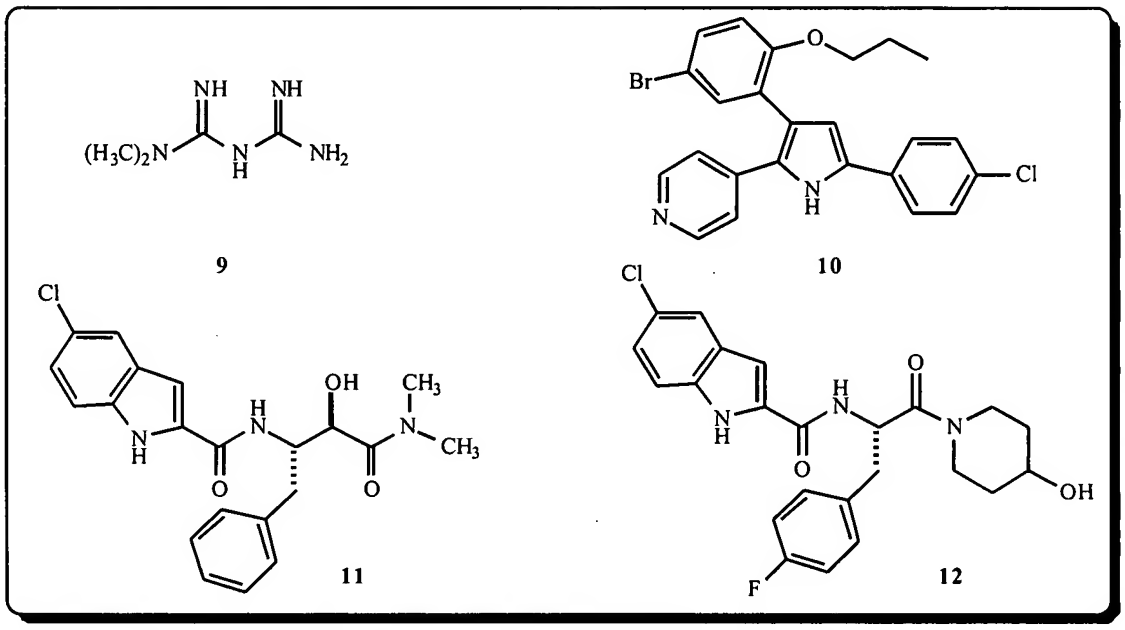
Metformin **9** remains a primary therapeutic option for Type 2 diabetics [36]. Metformin interferes with several processes linked to HGP (gluconeogenesis, glycogenolysis and their regulatory mechanisms), lowering glucose production and resensitizing the liver to insulin. Metformin reduces intestinal absorption of glucose, and increases glucose sensitivity, increasing peripheral glucose uptake and utilization. The precise molecular mechanism of action of metformin continues to be a subject of intense interest and has been reviewed extensively [37]. Unlike other anti-diabetics, metformin has been associated with significant weight loss. New studies have described the effectiveness of combination therapy using metformin and other standard anti-hyperglycemics for the maintenance of blood glucose levels [38].

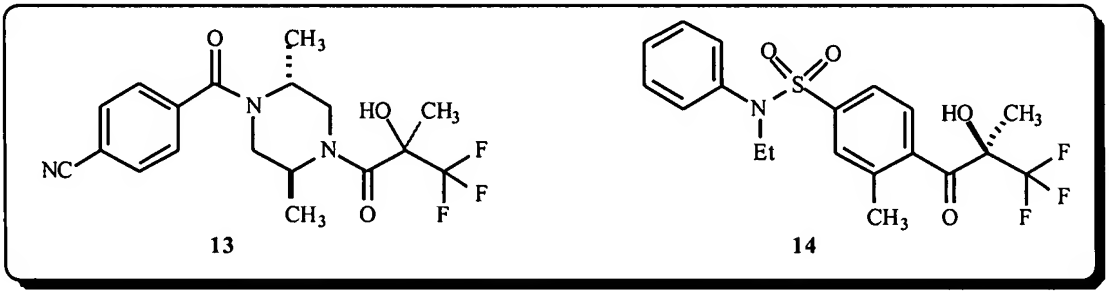
Glucagon Receptor Antagonists

Glucagon receptor antagonists have been the subject of a recent comprehensive review article [39]. Reports describing the discovery and optimization of potent, orally absorbed glucagon receptor antagonists, typified by L-168,049 **10** (K_b=25 nM, non-competitive with glucagon), have appeared. Optimization of the potency and selectivity of this series of compounds for glucagon receptor antagonism vs. inhibition of the Ser/Thr p38 (MAPKinase) was described in detail [40,41].

Glycogen Phosphorylase

Reduction of glycogenolysis by the inhibition of glycogen phosphorylase, the enzyme that releases glucose-1-phosphate from glycogen, is another approach to inhibiting HGP. CP-91149 **11** and CP-360626 **12** are potent inhibitors of human





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liver glycogen phosphorylase and also inhibit glucagon stimulated glycogenolysis in rat and human hepatocytes [42,43]. Oral administration of **11** to diabetic mice (25-50 mg/kg) resulted in blood glucose lowering of ~50% [42]. Chronic administration of **12** to diabetic *ob/ob* mice also significantly decreased elevated plasma triglyceride, cholesterol, insulin, and lactate levels [43]. CP-320626 was also found to lower plasma cholesterol in a non-glucose dependent manner by inhibiting cholesterol synthesis *via* direct inhibition of CYP51 (lanosterol 14α-demethylase) [44].

Pyruvate Dehydrogenase Kinase (PDHK) Inhibitors

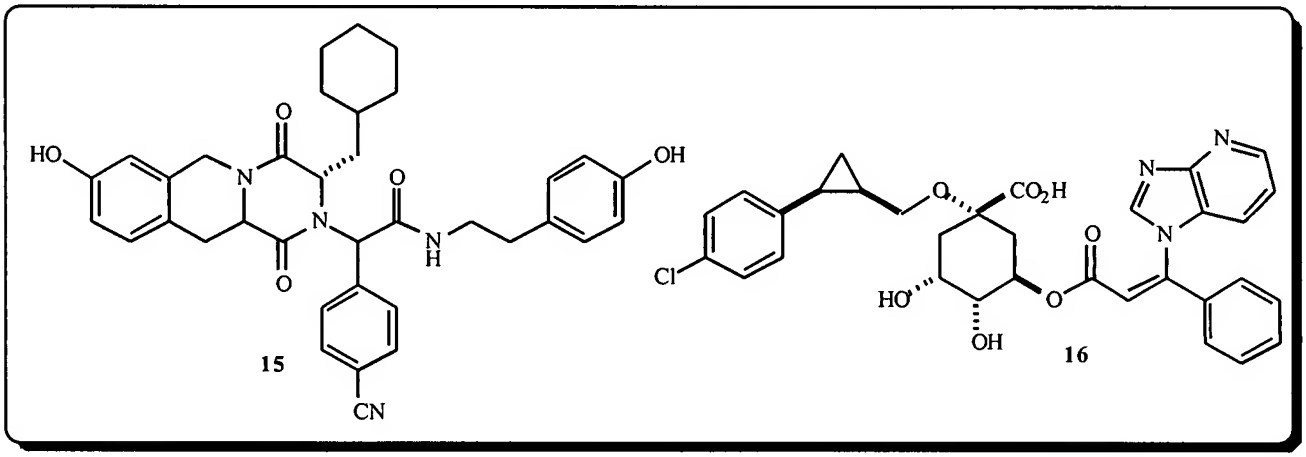
PDHK regulates pyruvate dehydrogenase (PDH), the enzyme which catalyzes the decarboxylation of pyruvate to acetyl-CoA. Activation of PDH increases oxidative glucose metabolism and reduced PDH activity has been linked to reduced glucose utilization in diabetes, as well as enhanced gluconeogenesis and impaired insulin secretion [45]. Dichloroacetic acid, an inhibitor of PDHK, among other enzymes, has been shown to lower lactate and glucose levels *in vivo* [46]. Two new classes of potent, specific PDHK inhibitors, represented by **13** and **14,** have been reported [45,47]. **13** has been shown to be orally available and while it significantly reduced lactate in normal, fasted rats, it did not lower blood glucose levels in diabetic animal models [48].

Fructose-1,6-bisphosphatase (FBPase) Inhibition

FBPase is an important enzyme in the glycolytic-gluconeogenic pathway and ensures a unidirectional flux from pyruvate to glucose, and also plays an essential role in the maintenance of normoglycemcia during fasting. Amide **15** has been shown to be a potent (IC_{50} = 0.37 μM) inhibitor of this enzyme [49].

Glucose-6-phosphatase (G-6-Pase) Inhibition

G-6-Pase is a multicomponent enzyme, resident in the endoplasmic reticulum (ER), that catalyzes the final step in both gluconeogenesis and glycolysis [50]. Inhibition of glucose-6-phosphate translocase, a component of G-6-Pase which



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permits entry of glucose-6-phosphate into the ER, is expected to modulate inappropriately high rates of HGP. **16** was shown to inhibit the multi-subunit G-6-Pase with an IC_{50} of 10 nM [50] by slowing the translocase function, inhibiting glucose output from rat hepatocytes, and significantly lowering circulating plasma glucose concentration in fasted rats and mice.

ENHANCERS OF INSULIN ACTION

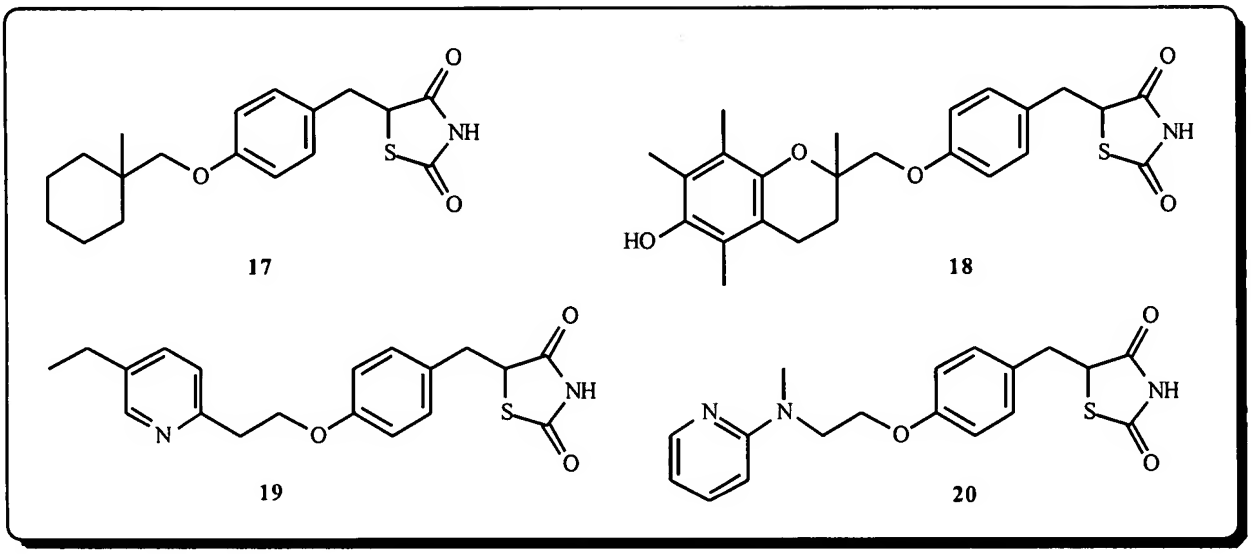
Ligands for Peroxisome-Proliferator Activated Receptor γ (PPARγ)

The role of the PPARγ nuclear receptor has expanded tremendously since its discovery as an orphan receptor over a decade ago [51-61]. PPARγ, once only associated with diabetes and obesity, has recently been implicated in the transcriptional control of a wide variety of cellular processes such as the cell cycle, lipid homeostasis, inflammation, carcinogenesis, and immunomodulation [54-61]. Recent research has indicated that PPARγ plays a major genetic role in efficient energy storage by controlling the "thrifty gene response", and is emerging as a central mediator connecting energy reserves and systemic homeostasis [51,52]. There are three identified isotypes of PPAR: α, β (also called δ or NUC1) and γ. The α and β forms will be discussed in a separate section of this review. PPARγ is expressed in appreciable amounts in adipose tissue, colon, retina, and to a lesser extent in muscle and liver. As part of the insulin signaling cascade, PPARγ up-regulates proteins required for the metabolism of glucose and lipids. Ligands of PPARγ activate the glucose transporter gene resulting in increased production of glucose transporters (eg, GLUT4) in muscle, liver and fat [62]. Surprisingly, very little has been published concerning the genetic and biological connection between PPARγ and GLUT4. PPARγ also has an effect on leptin production in adipose tissue which is supportive of PPARγ's role in promoting lipogenesis. PPARγ negatively controls leptin expression [63]. Interestingly, PPARγ and leptin gene expression are both increased upon feeding which may lead to a reduction in food intake. This is supported by a study with *PPARγ*+/– mice [64].

Thiazolidinedione (TZD) agonists of PPARγ have an effect on glucose disposal which is typically thought to occur in the muscle, but PPARγ is expressed in very small amounts in this tissue. The glucose disposal may be the effect of PPARγ up-regulation of uncoupling proteins (UCP). In several skeletal muscle and adipose tissue cell lines, PPARγ activation by TZDs stimulated UPC2 expression [65]. The regulation of energy metabolism in relation to PPARγ, for example ATP synthase (F0F1), UCPs, and reactive oxygen species (ROS), is mediated by PPARγ coactivator-1 (PGC-1) [66]. PGC-1 is expressed in all of the same tissues as PPARγ. When PGC-1 is bound to PPARγ, a conformational change occurs which allows the binding of SRC-1 and CBP/p300, vastly increasing transcriptional activity [67] PGC-1 not only plays a coordinating role in the thermogenic activity of brown fat adipose tissue, but also in the control of mitochondrial genes encoding fatty acid β-oxidation (FAO) enzymes when bound to PPARα [68]. Thus, PGC-1 also plays a part in fatty acid oxidation and lipolysis. It has also been shown that activation of PPARγ leads to expression of c-Cbl associated protein (CAP) [69]. CAP is a protein found in insulin sensitive tissues, and is involved in the insulin-stimulated phosphorylation of c-Cbl which may play a role in glucose homeostasis. Thus, PPARγ agonists reduce hyperglycemia without increasing the amount of insulin secretion by increasing cellular glucose uptake, reducing cellular glucose production, and increasing insulin sensitivity in resistant tissues.

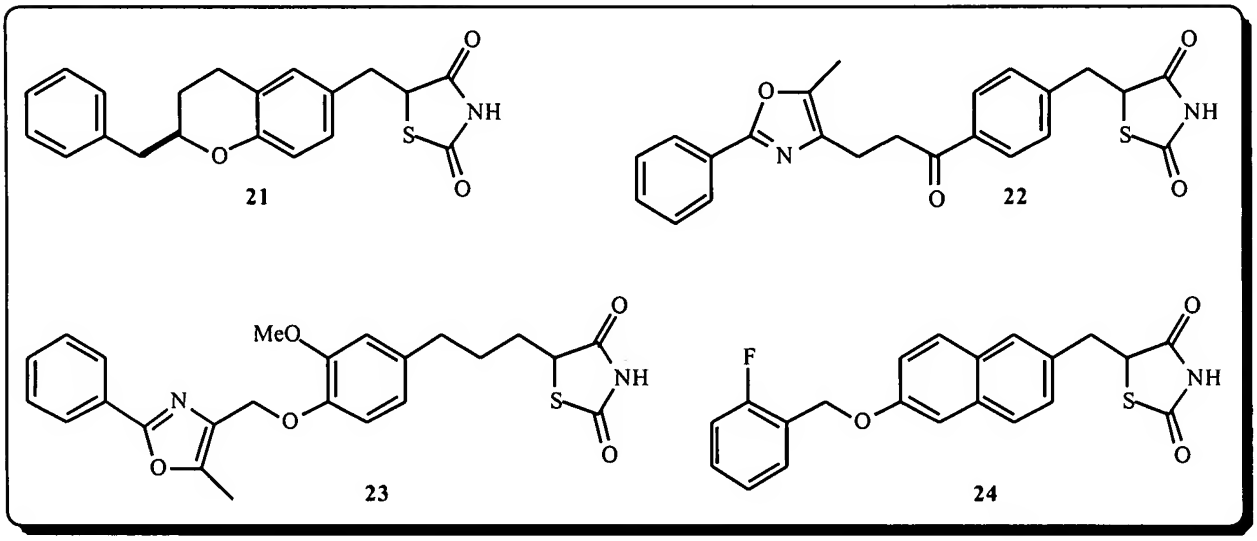
Thiazolidinediones (TZDs)

The mode of action and clinical benefits of the TZDs have been recently reviewed [70-75]. A mutation in the encoding gene for PPARγ, which disrupts blood glucose and blood pressure control, provides further evidence that this receptor is the target of the TZDs [76]. The first in the class was Ciglitazone (**17**) which was discontinued during early clinical trials. Troglitazone (Rezulin, **18**), which showed an improved oral glucose disposal, was launched in 1997 and has been well reviewed [77-80]. Because of concern over related liver



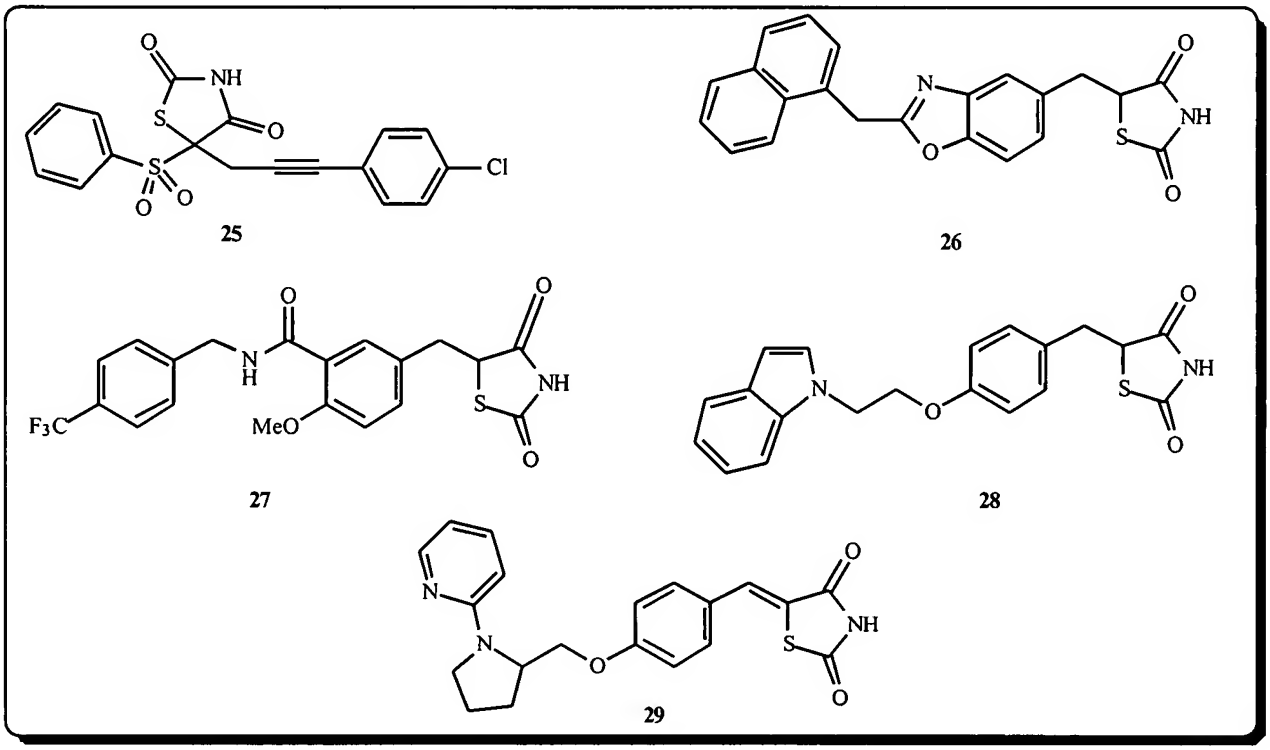
failure, troglitazone was withdrawn from the European market in 1998 [75,81,82]. The liver dysfunction is idiosyncratic and effects approximately 2% of patients in these trials. The cause of the hepatotoxicity may be related to activation of gene expression in the liver, toxic metabolites, or drug-drug interactions. It has been shown that troglitazone (**18**) can activate pregnane X receptor (PXR), a newly isolated orphan nuclear receptor [83]. The PXR receptor regulates transcription of cytochrome P450 3A4 (CYP3A4) which is the cytochrome responsible for the oxidative metabolism of the majority of xenobiotics in the liver. In the US, the FDA has restricted troglitazone's (**18**) use and recommended frequent monitoring of liver enzymes. However, the FDA did approve troglitazone (**18**) as a combination therapy with sulfonylureas and metformin which gives improved glycemic control in type II diabetes [84-86]. Troglitazone (**18**) has also been linked to increased levels of lipoprotein(a) which is a risk factor in coronary heart disease,[87] but also cardioprotective effects based mostly on slightly reduced blood pressure [74,88].

Pioglitazone (Actos, **19**) and rosiglitazone (Avandia, **20**) were both launched in the US in 1999. Pioglitazone (**19**) in doses of 30 mg and 45 mg resulted in reductions in mean HbA1c of 2.3% and 2.6% from baseline in previously untreated patients [89,90]. Oxigenated metabolites of pioglitazone are slightly less potent than the parent [91]. Rosiglitazone (**20**) given 4 mg bid results in a 1.5% reduction in HbA1c relative to placebo, and reduces HbA1c 1.2% over metformin alone [92-94]. Both compounds have similar side-effect profiles, although there have recently been reports of idiosyncratic hepatotoxicity in a few patients receiving rosiglitazone (**20**) [95-97]. Other TZDs in various stages of clinical development include englitazone (**21**) which may have an effect on gluconeogenesis in Zucker rat livers and has been used to probe the insulin signaling pathway *in vitro*,[98,99] and darglitazone (**22**) which has been shown to increase body weight in fatty Zucker rat due to increased feeding,[100] and **23** [101]. MCC-555 (isaglitazone, **24**) has been shown to prevent or delay onset of diabetes and has a positive effect on free fatty acids and triglycerides in Zucker rats. This TZD is notable because it breaks from the rule that PPARγ binding correlates with anti-diabetic activity. MCC-555 (**24**) has low binding potency, but is a highly effective PPARγ activator [102-105]. This may be an indicator that PPARγ agonism is a more complicated process than first suspected. Some PPARγ agonists may exert a portion of their anti-diabetic effect through activation of other PPARs or through activation of the PPAR/RXR heterodimer as discussed below. It would certainly change the way TZDs and PPAR agonists are assayed and developed, if it is found that there is tissue- or ligand-specific gene modulation. Of



course other mechanisms of action such as active metabolites have not been ruled out.

Several TZDs were withdrawn from clinical development including BM-13.1246 [106] and indolylthiazolidinedione [107]. While some have entered preclinical development, such as the new preclinical (**25**) [108], T-174 (**26**) [109] and KRP-297 (**27**) which has an amide bond in the bridge moiety [110]. While TZDs are typically selective for PPARγ, **27** has potent agonist activity against PPARα and γ [111]. A novel indole compound DRF-2189 (**28**) derived from SAR around known clinical TZDs was shown to have similar glucose disposal properties (~50% reduction of blood glucose with 10 mg/Kg/day for 14 days in *db/db* mice) as its parent rosiglitazone [112,113]. A more recent pyrrolidine **29** analogue dosed at 30 mg/Kg/day *po* x 14 days gives a 73% reduction in blood glucose with a 68% reduction in triglycerides in *ob/ob* mice [114]. Two compounds in this study showed good glucose disposal, but neither showed

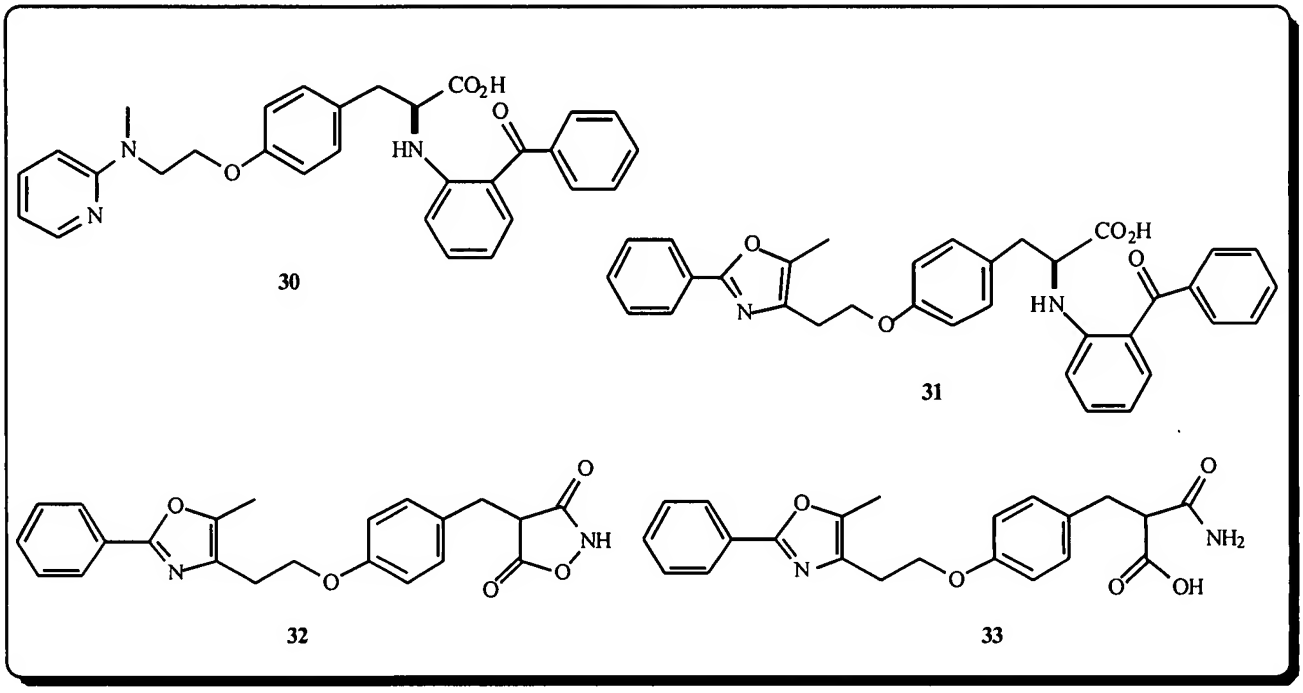


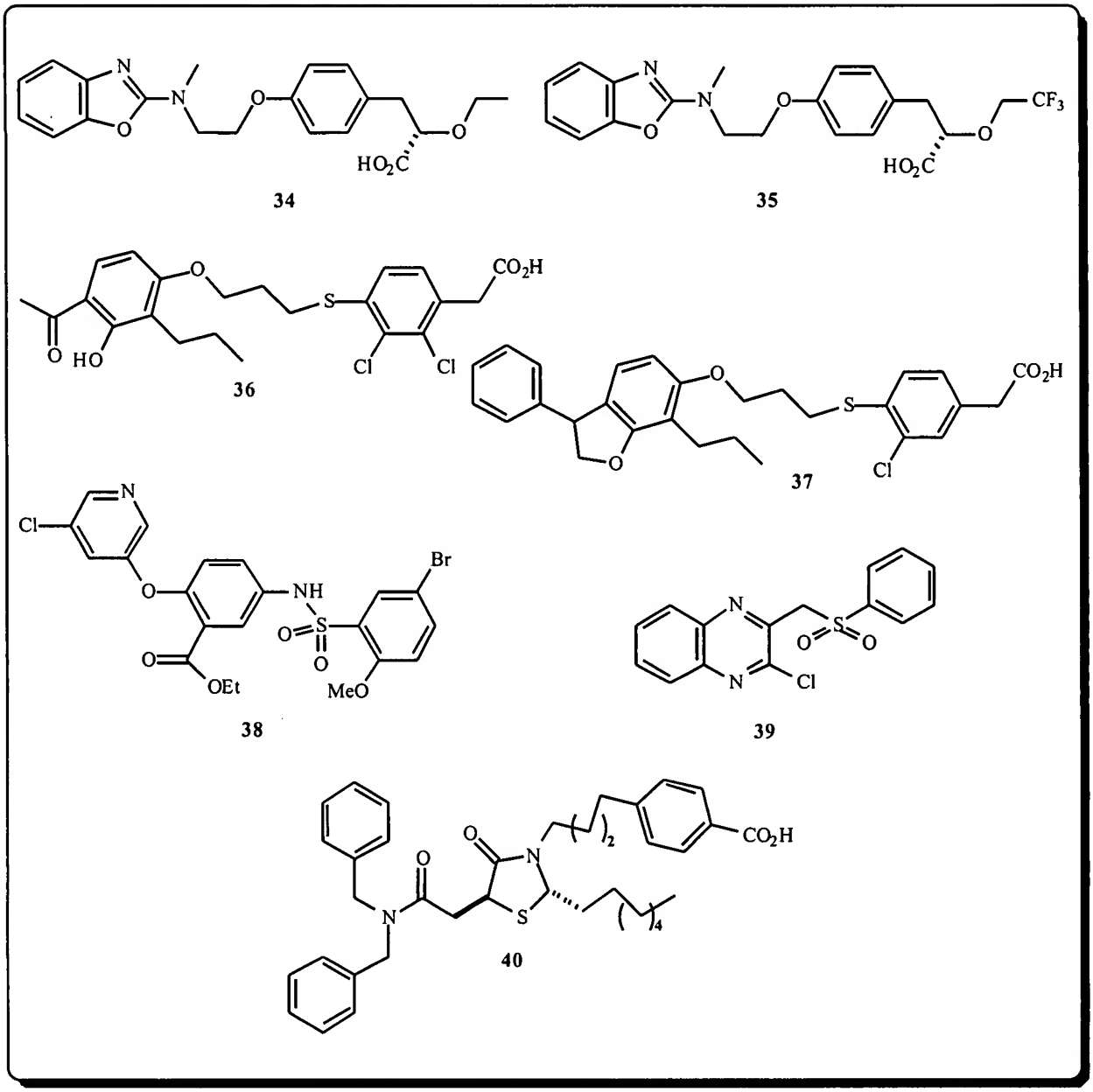
significant transactivation of PPARα or PPARγ. Interestingly, other analogues in the series which did show transactivation of PPARγ were poor controllers of blood glucose. There is still much to discover about the biology of the PPARγsignaling system.

Non-thiazolidinediones (non-TZDs)

There are now a variety of potent non-TZD PPARγ binders which exhibit antidiabetic activity *in vivo*. Non-TZDs in development are GW-1929 (**30**) [115,116], GI-262570 (**31**) [117], GW-409544 [118], JTT-501 (**32**) [119], and YM-440 [120]. Interesting, JT-501 activity is likely to be in close association with its hydrolysis metabolite **33** [121]. JT-501 (**32**) is also reported to moderately activate PPARα at therapeutic concentrations [122]. GW-1929 (**30**) is approximately 100-fold more potent in rats than troglitazone in glucose lowering based on serum drug levels [123]. The 4-hydroxyphenylalkanoic acid GI-262570 (**31**) has entered Phase III. Analogues in this series are highly selective toward PPARγ and are highly potent *in vivo*; some having sub-nanomolar activity against human PPARγ. Related L-tyrosine analogues were synthesized on resin [124]. The (*S*)-enantiomers of TZDs are known to bind PPARγ with much higher affinity than their antipodes [125]. Unfortunately, the TZD's readily racemize *in vivo* leaving half of the drug inactive. In this respect, the acyclic non-TZDs may have an advantage since they are less susceptible to racemization. Chiral analogues in the GW series confirm that the (*S*)-enantiomers of these non-TZDs also have a greater binding affinity and agonist activity for PPARγ [123].

In adipocyte differentiation assays, the (*S*)-enantiomers of a series of α-alkoxy propanoic acids (SB-236636, **34**) were revealed to have higher binding affinity and more potency than the (*R*)-isomers [126]. The (*S*)-enantiomer of SB-219994 (**35**) has a 770-fold better binding to PPARγ then its antipode which is reflected in the (*S*)-isomer's 100-fold higher efficacy as an antidiabetic [127]. Some novel PPARγ agonist structures have been found such as the phenylacetic acid compounds **36** [128] and **37** which reduces hyperglycemia by 65% at 30 mg/kg/day in Zucker rats [129]. Other novel non-TZDs include an arylsulfonylamide obtained from high-throughput screening **38** [130] and the quinoxaline L-764406 (**39**) which is a PPARγ partial agonist [131]. Also a partial agonist, GW-0072 (**40**) had only about 20% of the efficacy of rosiglitazone in transactivation assays and was an inhibitor of adipocyte differentiation





[132]. Molecules with novel agonist or partial agonist activity may be valuable for probing the complex molecular regulation of the PPARs.

Other PPAR Isomers in Diabetes Therapy

PPARα and PPARδ agonists are now being investigated for the prevention of diabetic risk factors and treatment of complications. For example, PPARα is implicated for the treatment of obesity, lowering serum triglycerides, raising HDL cholesterol (HDLc), and preventing lesions in arterial walls. While PPARγ is produced mostly in adipose tissue, PPARα is expressed in high levels in the liver, heart, and muscle [133]. Notably, PPARα is found in tissues which are more metabolically active than PPARγ. Obesity is one of several notable risk factors associated with diabetes which may be ameliorated by treatment with PPARα agonists such as fibrates. In rats treated with fibrates, weight loss was observed without changes in food intake [134], which may be related to increased activity of mitochondrial uncoupling proteins (UPCs) [135]. For lipid-lowering activity, it is suggested that fibrates and the thioisobutyric acid GW9578 repress hepatic

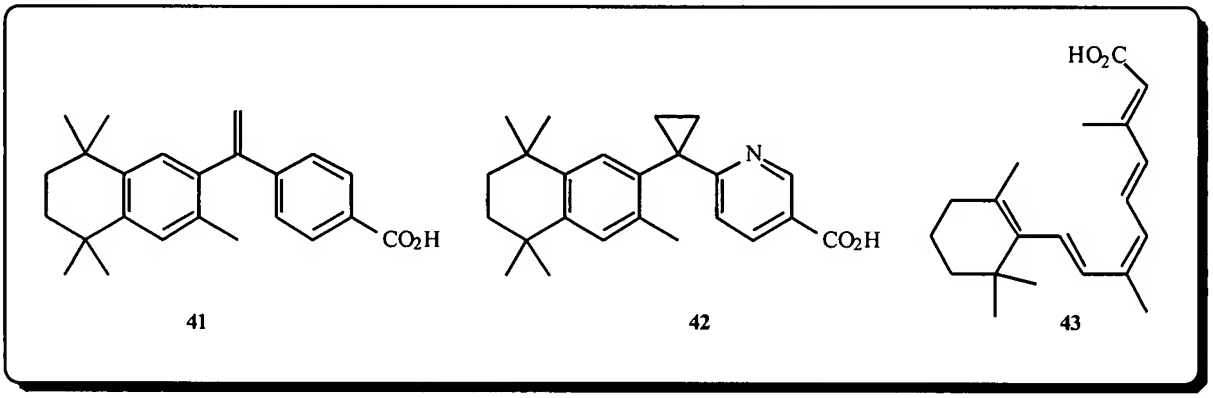
apoC-III expression (an inhibitor of VLDL clearance) and increase lipoprotein catabolism [136]. PPARα agonists may even help prevent atherosclerosis by down-regulating the monocyte adhesion molecule VCAM-1 [137].

PPARδ (previously reported as PPARβ) has been implicated in lipid and cholesterol homeostasis. In *db/db* mice, plasma cholesterol was raised by treatment with 30 mg/kg L-165041. Since L-165041 is only a modest PPARδ selective agonist, the pharmacological activity may be in part due to other PPARs. The fact that neither plasma glucose or triglyceride levels were effected indicates that the *in vivo* activity is due mostly to the activation of PPARδ, not PPARγ. In the same study, a related set of dual PPARδ and PPARγ agonists were evaluated. Interestingly, the compounds showed dual activity including an increased plasma glucose disposal [138]. This suggests that specifically tailored compounds possessing agonist activity among more than one PPAR may hold promise for simultaneously treating a host of metabolic dysfunctions.

Rexinoids and the Retinoid X Receptor (RXR)

The retinoid X receptor (RXR) is a ligand activated transcription factor which forms a heterodimeric complex with PPARγ. Specific ligands of the RXR receptor are designated rexinoids. The RXR/PPARγ complex regulates the expression of genes which control lipid and carbohydrate metabolism as well as the differentiation of adipocytes. The biology and applications of RXR and related receptors has been recently reviewed [139]. Treatment of patients with an RXR agonist produces a similar response as treatment with a PPARγ agonist: insulin sensitivity is improved, glucose and lipid uptake is increased, and energy utilization is improved [140]. Further investigation has shown that certain rexinoids function as RXR heterodimer-selective agonists. For example, rexinoids targretin (LGD-1069, **41**) and LG-100268 (**42**) selectively activate the RXR/PPARγ and RXR/LXR (orphan receptor) heterodimers, but not RXR/RAR (retinoic acid receptor) or RXR/TR (thyroid hormone receptor) complexes. In diabetic mouse models, these rexinoids are insulin sensitizers and can decrease hyperglycemia, hypertriglyceridemia and hyperinsulinemia. Combination treatment with rosiglitazone and rexinoids enhances the antidiabetic effect presumably by increased activation of RXR/PPARγ [141].

The activation of RXR/PPARγ was found to stimulate genes (FATP-1 and ACS) implicated in the metabolism of lipids. Even though they act on the same receptor complex, rexinoids may modulate insulin sensitivity differently than PPAR agonists [142]. Use of retinoid compounds like 9-cis retinoic acid (**43**) may provide a treatment for NIDDM or delay onset of the disease in at-risk patients [143]. Pursuant of this idea, a series of cyclopentylidene rexinoid analogues (**44**) with high selectivity toward RXR are reported to show reduced blood glucose and triglyceride levels [144]. A novel series of oxime and oxime derivative rexinoids (**45**) are highly specific activators of the RXR:PPARγ complex and induce differentiation of 3T3-L1 preadipocytes to adipocytes [145]. The 2-



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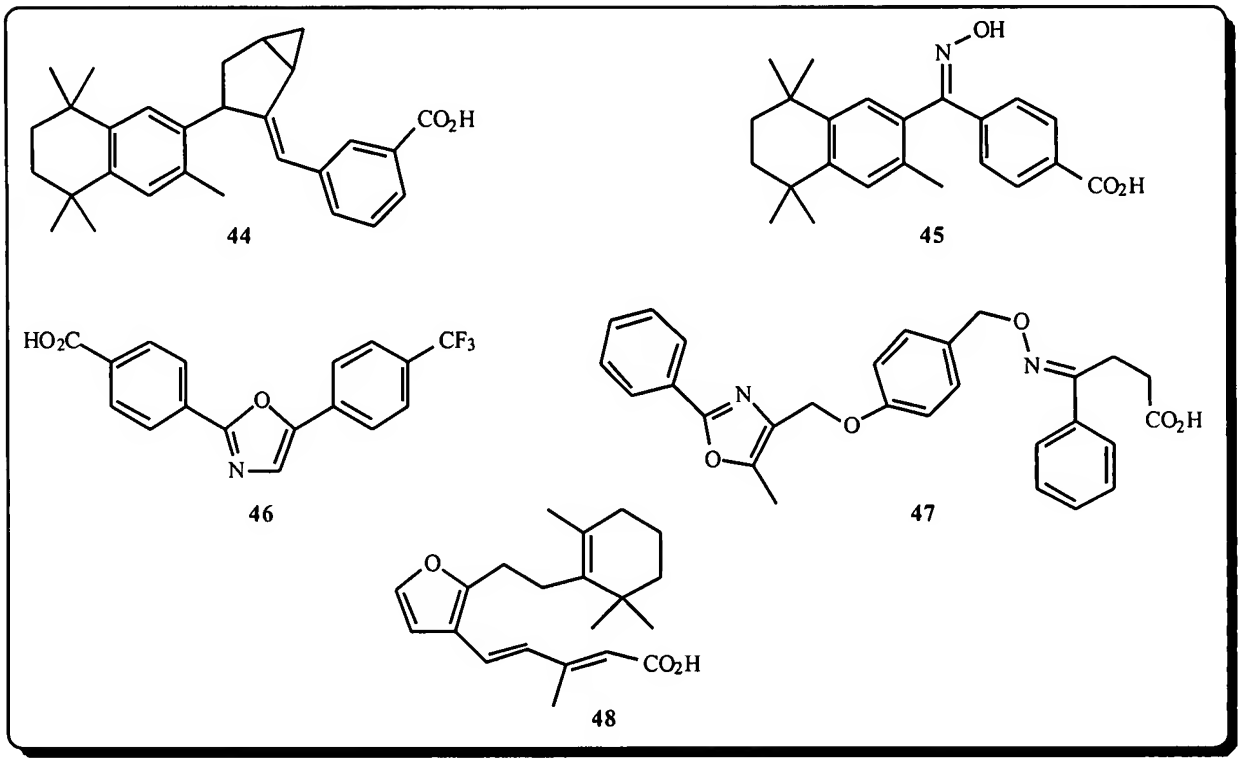
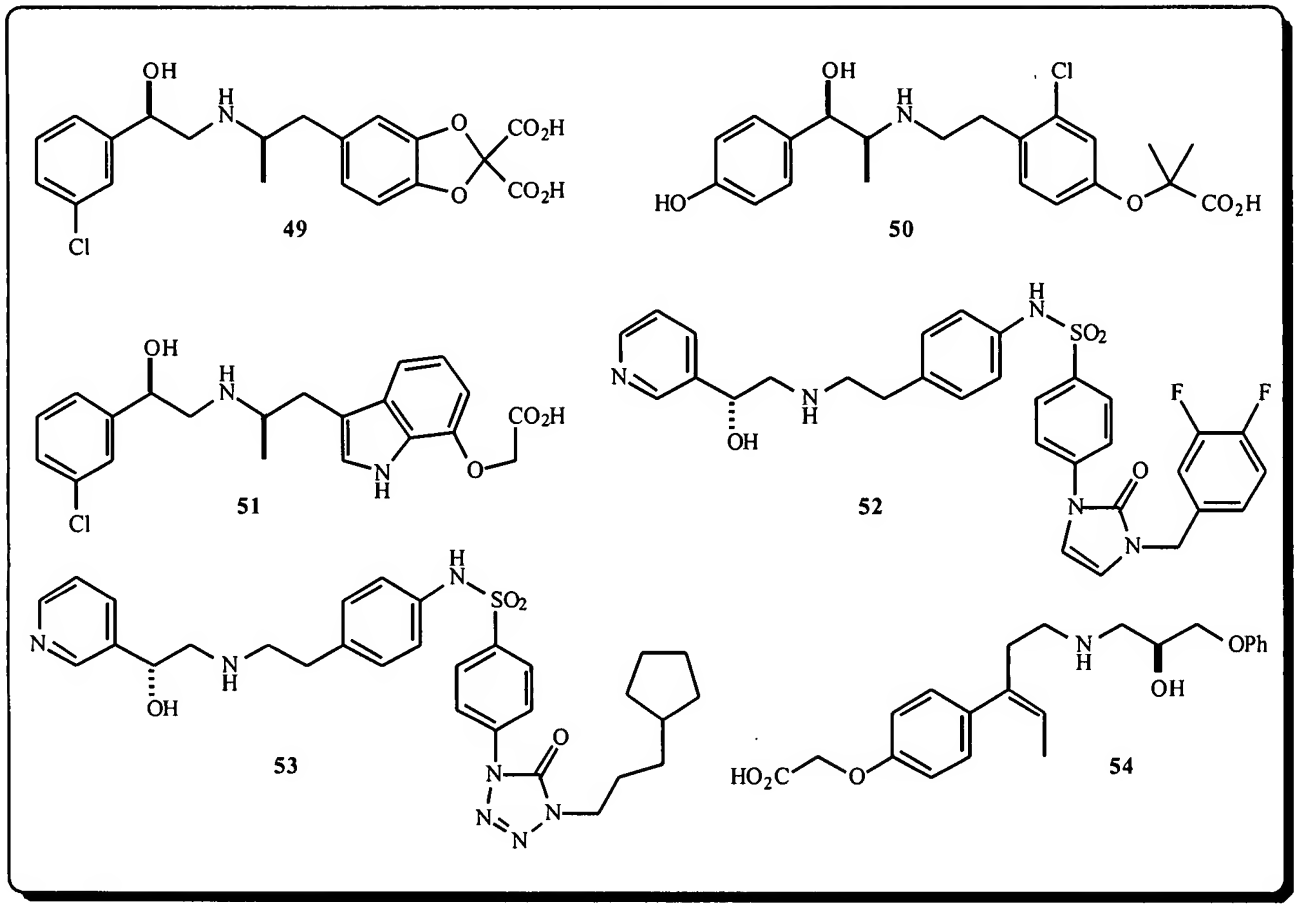


phenyloxazole compound (**46**) and related compounds were reported to lower hyperglycemia by 36% to 54% and hypertriglyceridemia by 35% to 82% when given orally to KKAy mice for 4 days. These compounds also showed an excellent selectivity toward PPARγ/RXRα heterodimer [146]. The 2-oxazole compound **47**, as the free acid, lowered blood sugar by 51% when feed to diabetic mice [147]. A few conformationally constrained analogues of retinoic acid **48** are being investigated as specific ligands for RXR [148].

Armed with well characterized RXR and PPARγ agonists, the pathology of diabetes and the biological contribution of RXR and PPARγ are under investigation. In *db/db* mice treated with LG100268 or rosiglitazone, blood glucose and fibrinogen (cardiovascular risk factor) were reduced. Interestingly, both agonists were effective in increasing insulin formation in β cells. This study indicates that RXR and PPARγ agonists may protect β cell function. Treatment with both agents improved all measured diabetic endpoints better than each alone. The RXR agonists seem to have a greater effect on liver metabolism than the PPARγ agonists causing peroxisome accumulation and hepatomegaly [149]. There are also indications that RXR agonists may lead to changes in thyroid metabolism as seem in clinical trials of LGD-1069 (**41**) [150]. While these specific agonists of RXR and the RXR:PPARγ heterodimer seem like a useful emerging therapy for NIDDM, there is still doubt about their toxicity. It is also too early to tell what the effect of RXR agonists will be when combined with specific PPAR isozyme agonists. The efficacy in diabetic models and from cancer patients is clear and promising, but whether rexinoids will be used safely as a monotherapy or in combination with a PPAR agonist remains to be determined.

β_3 Adrenergic Receptor (b_3AR) Agonists

Agonists of β_3ARs increase the release of energy stores as heat in brown adipose tissue, increase lipolysis in white adipose tissue, suppress food consumption, suppress leptin gene expression and serum leptin levels. Uncoupling proteins (UCPs) in the inner mitochondrial membrane generate heat without effecting other energy-consuming processes. After treatment with the β_3AR agonist CL316,243 (**49**) (0.2 mg/kg/day), KK-Ay mice showed increased UCP expression in



adipose tissue, but not in muscle and heart. Further more, the concentration of plasma insulin and free fatty acids were significantly reduced [151]. Unfortunately, the response to β_3AR agonists seems to decrease with age which corresponds with the increase of obesity in mature animals [152]. CL316,243 (**49**) has also been prepared in ester form to improve its bioavailability [153]. Several novel β_3AR agonist structures are indicated as antidiabetics including 2-methylpropionic acid derivatives (**50**) [154], AJ-9677 (TAK-677, **51**) which is in Phase 1 [155], and a selective series of indole-5-sulfonamide agonists [156]. Imidazolidinone L-766,892 (**52**) gives a dose-dependent lipolytic response in rhesus monkeys with minimal effects on heart rate [157]. Related partial agonists L-760,087 and L764,646 are also highly selective for β_3AR [158]. L-770,644 (**53**) is a full agonist in rhesus monkeys (0.2 mg/kg) with good in vivo properties [159]. The β_3AR agonist SWR-0342SA (**54**) is highly selective, and reduces blood glucose by 46% and serum insulin levels in KK-Ay mice [160].

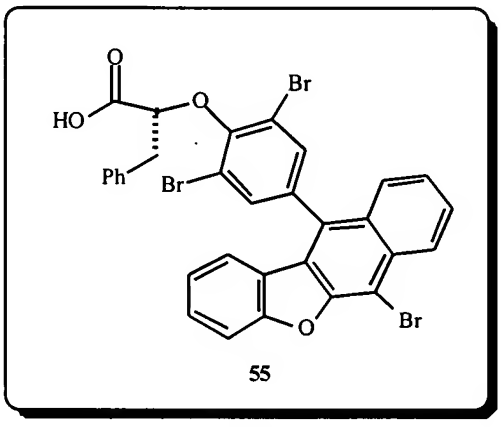
Protein Tyrosine Phosphatase-1B (PTP-1B)

It has long been thought that protein tyrosine phosphatases, such as protein tyrosine phosphatase-1B (PTP-1B), have played a role in the negative regulation of insulin signaling and are involved in the insulin resistance associated with Type 2 diabetes [161]. Kennedy and Ramachandran have recently demonstrated that mice lacking the PTB-1B gene have enhanced insulin sensitivity [162]. Treatment of these mice with insulin results in prolonged and increased protein phosphorylation of the insulin receptor in liver and muscle tissue, suggesting that PTP-1B is critical in the dephosphorylation of the activated insulin receptor and that inhibition of this enzyme would be an excellent strategy for the treatment of Type 2 diabetes. An interesting phenotype of these PTP-1B deficient mice is their resistance to diet-induced obesity and increased energy expenditure [163].

The development of selective inhibitors of PTP-1B is important to help clarify the role that

this phosphatase plays in insulin signaling. Progress is being made in the development of inhibitors of PTP-1B, but selectivity vs. other, related phosphatases is still a major issue. One of the early inhibitors of PTP-1B is vanadate, which is a reversible, non-selective inhibitor of PTP-1B, with affinity in the micromolar range [164]. Vanadate's inhibitory activity has been shown to depend upon the redox state of the cell [165]. This redox dependence may contribute to the useful therapeutic index of this agent, and vanadate has been used in several clinical trials, demonstrating efficacy in improving the insulin sensitivity in Type 2 diabetes patients [166]. Analogues of vanadate with increased cell permeability and different complexation properties have been studied extensively; this area has been extensively reviewed [167].

Peptide sequences around phosphotyrosine play a critical role in modulating the affinity of the substrate peptide towards PTP-1B in particular and phosphatases in general [168]. Extensive peptide SAR studies been done in order to better understand the substrate selectivity of PTP-1B and subsequently to use this information to find potent, selective phosophorylated peptide inhibitors of PTP-1B [169]. In addition, protein tyrosine phosphatase peptide substrate analogs in which the cleavable linkage of a phosphotyrosine has been replaced by a hydrolysis resistant bond such as O-S (sulfate), [170] O-P-S (thiophosphate), [171] C-P (phosphonate),[172] and O-C (malonyl) [173] have also yielded extremely potent PTP-1B inhibitors, with affinities approximately the same as the parent phosphotyrosine peptides. These compounds all suffer, however, from the fact that they are peptide derived phosphates and phosphonates, and are not good drug candidates due to their unfavorable physical properties.



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The development of non-peptidic PTP-1B inhibitors has been somewhat slower than the peptides described earlier. Aryl phosphonates have been shown to inhibit PTP-1B, but the potency of these agents is only moderate, though these agents have been shown to have selectivity *vs.* other phosphatases [174]. Recently, however, a series of potent, non-peptidic PTP-1B inhibitors, such as **55** have been disclosed. The biaryl derivative **55** has an IC_{50} of ~115 nM for the inhibition of PTP-1B and has been shown to significantly lower blood glucose and insulin in a *ob/ob* diabetic mouse model [175]. These seminal studies provide the first hope that a non-peptide, small molecule inhibitor of PTP-1B can be used for the treatment of Type 2 diabetes, and should serve as an important guide for the development of even more potent agents.

Glycogen Synthase Kinase-3 (GSK3)

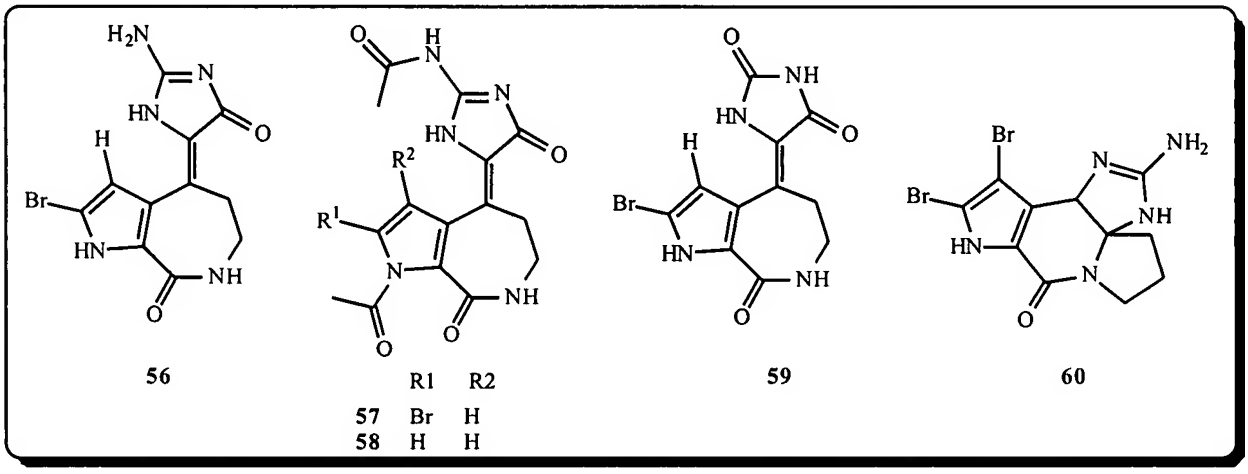
Recently glycogen synthase kinase-3 (GSK3), a critical kinase in the insulin signaling pathway, has received attention as a potential target for the development of antidiabetic therapies [176]. GSK3 is a vital regulatory serine/threonine kinase which inactivates glycogen synthase (GS) *via* phosphorylation [177]. Details of glycogen metabolism in the liver have been reviewed [178]. Two isoforms have been identified, α and β, which are coded by specific genes and independently phosphorylate GS [179]. Through the action of insulin, GSK3 is inactivated while glycogen bound protein phosphatase 1 (PP1G) is activated. PP1G dephosphorylates and activates GS for glucose disposal [180]. Therefore, inhibitors of GSK3 stimulate glycogen synthesis activity, and possibly other insulin-dependent blood glucose lowering events such as the ras and p70 S6 kinase pathways. Regulation of GSK3 has been proposed as one contributing factor in the progression of diabetes. For example, GSK3 is overexpressed in type 2 diabetic muscle, but not in normal-weight

nondiabetic or obese nondiabetic subjects. Further, as the activity of GSK3 increases in diabetic muscle, the tissue's sensitivity toward insulin decreases. GSK3 protein levels and total activities are inversely correlated with both GS activity and maximally insulin-stimulated glucose disposal [181]. Overexpression of GSK3 in CHO cells, which also express insulin receptor (IR) and insulin receptor substrate 1 (IRS-1), lead to a marked reduction in insulin action [182]. GSK3 activity was also examined in mice susceptible (C57BL/6J) to or resistant (A/J) to diet-induced obesity and diabetes. The GSK3 activity in susceptible (C57BL/6J) mice was 2-3 times higher in adipose tissue compared to A/J mice. Also, the GSK3 activity increased two fold in those C57BL/6J mice fed a high-fat diet in contrast to the low-fat diet C57BL/6J control mice. This mouse study implicates GSK3 in the susceptibility and progression of diet-induced diabetes [183].

The role of GSK3 in glucose metabolism has been probed by several direct methods. The contribution of GSK3 in insulin-stimulated glucose disposal was examined in 3T3-L1 adipocytes using adenovirus technology. Three types of GSK3 were introduced into separate cell lines: wild-type (WT-GSK), catalytically inactive (KM-GSK), and uninhibitable (S9A-GSK) forms of GSK3β. WT-GSK, but not KM-GSK, reduced basal and insulin-stimulated GS activity. S9A-GSK decreased IRS1-associated phosphatidylinositol 3-kinase and GS activity, but only weakly affected Akt/PKB phosphorylation. There was no effect on GLUT4 translocation. In the same study, GSK3β activity was inhibited with lithium. There was no effect on GLUT4 translocation or pp70 S6 kinase activity. The increase in glucose transport with lithium was insensitive to wortmannin which should act upstream of GSK3 on phosphatidylinositol 3-kinase (PI3K). These data suggest that GSK3β stimulates production of glycogen, but does not contribute to glucose transport [184].

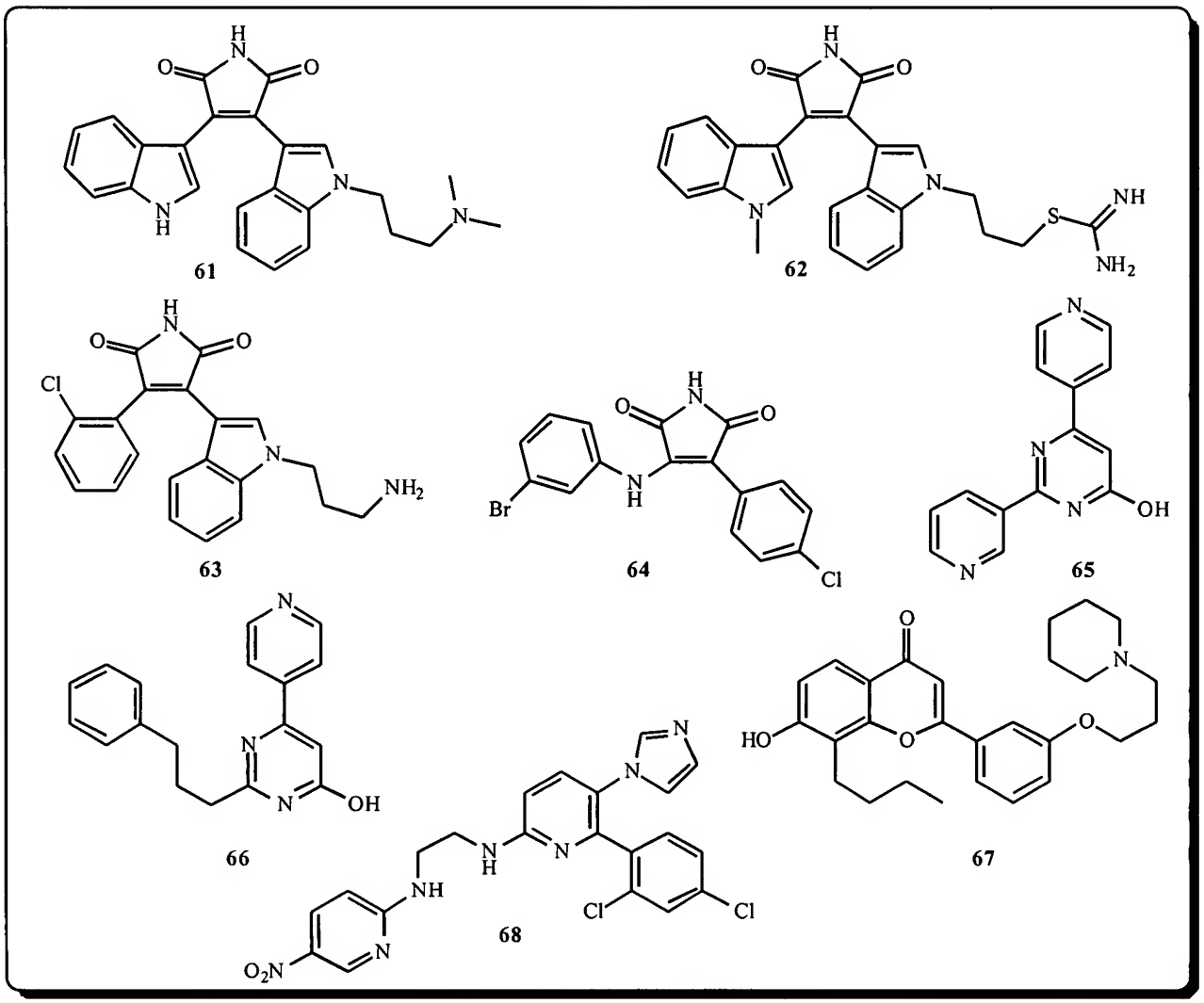
Lithium appears to inhibit GSK3 in a non-competitive manner, and shows some ability to increase glucose disposal [185]. GSK3β is inhibited by hymenialdisine (**56**) (a marine sponge natural product) with an IC_{50} of 10 nM *in vitro*. Unfortunately, the inhibition was not specific for GSK3, but showed activity toward CDK1/cyclin B, CDK5/p25, and CK1. The inhibition was found to be competitive with ATP with an apparent K_i of 50 nM. Other related naturally occurring and chemically modified small molecules also inhibit GSK3; including diacetylhymenialdisine (**57**) (IC_{50} = 160 nM), diacetyldebromohymenialdisine (**58**) (IC_{50} = 600 nm), axinohydantoin (**59**) (IC_{50} = 3 uM), and dibromocantharelline (**60**) (IC_{50} = 3 uM) [186].

The known protein kinase C (PKC) inhibitors bisindolylmaleimide I (GF-109203x, **61**) and IX (Ro 31-8220, **62**) were found to be potent inhibitors of GSK3 when tested in cell lysates (IC_{50}'s = 360 nM and 6.8 nM respectively). The activity of these PKC inhibitors against GSK3 might help explain observations of the insulin-like



effects on glycogen synthesis activity [187]. Similar maleimide or carbazole compounds **63** and **64** were reported to inhibit GSK3 *in vitro* [188]. The *in vitro* and whole cell activity of these compounds was described in a recent publication. The authors note that selected GSK3 inhibiting compounds disclosed in their patents stimulated glycogen synthesis in human liver cells [189]. Although not specifically for treating diabetes, several patents were published which describe potent pyrimidine **65** and **66,** and benzopyran-4-one compounds **67** as novel inhibitors of GSK3 [190]. Potent pyrimidine- and pyridine-based compounds which inhibit human GSK3β were shown to improve glucose disposal and lower plasma insulin when given orally to *db/db* mice [191]. The reported efficacy of specific and potent GSK3 inhibitors in a diabetic mouse model lends credibility to this novel insulin-sensitizing kinase target as a focus of drug discovery. Further, the mechanism of GSK3 action on GS should not lead to hypoglycemia because of the maximal processing speed of GS, GS's dependence on UDP-glucose concentration, and other regulatory feedback pathways. A GSK3 inhibitor should act synergistically with other marketed drugs, and may hold advantage over some as a mono-treatment. More publications on the medicinal chemistry and pharmacology of GSK3 inhibitors are anticipated as programs mature and progress into clinical trials.

From the vantage point of developing small molecule effectors of insulin signaling, kinases are attractive targets because a good therapeutic effect is caused by competitive inhibition with ATP or a



protein substrate. It is much more difficult to elicit a positive metabolic effect with a small molecule by directly activating a kinase or inducing protein expression. In the insulin signaling cascade, GSK3 is a fairly optimal target since it is one of the last proteins in the cascade, acts proximally to glycogen production, has few known substrates, and is not likely to cause acute toxicity. There are several other kinases which have a positive effect in insulin signaling in the same pathway as GSK3, but are probably not as well suited for development of a diabetes drug, such as PI3K, PDK1, Akt1/PKB, PKC, and PP2A. PP2A1 (a Ser/Thr-specific phosphatase) dephosphorylates and activates GSK3. In vitro, insulin stimulation of GSK3β expressing 293 cells leads to 60% inhibition of GSK3 activity. Incubation of the stimulated cells with PP2A1 leads to a complete reversal of the GSK3β inhibition. In the same study, coexpression of GSK3β in 293 cells with either Akt/PKB or PDK1 (the upstream activator of PKB) simulated the insulin-induced phosphorylation of Ser-9 and inactivation of GSK3β. Thus, small molecule inhibitors of PP2A1 might be a viable way to maintain GS activation [192]. Unfortunately, PP2A1 dephosphorylates many targets, and holds the potential for serious toxic side effects in animals. PI3K, PDK1, Akt1/PKB, PKC, p90rsk, and PP1 are all activated in response to insulin-stimulation. PI3K, PDK1, Akt1/PKB, and PKC are all up-stream of GSK3, and act to block the action of GSK3. PP1 and p90rsk both act to increase the activity of GS in response to insulin [193]. Since the inhibition of these enzymes would lead to a reduction in glycogen formation, the only way to increase GS activity targeting these enzymes is by using gene therapy or screening for an up-stream small molecule activator or expression inducer. A method for assessing the phosphorylation state of Akt1/PKB to identify possible antidiabetic agents has been patented [194].

Miscellaneous Enhancers of Insulin Action

The novel hypoglycemic agent SDZ PGU 693 (**69**) was found to induce hepatocellular hypertrophy and a complex change in both cytochrome and fatty acid metabolism genes [195]. The general pharmacology and toxicology of S-15261 has been published, although the mechanism of action is still not fully understood [196]. The mechanism of insulin sensitization of the benzazine derivative (**70**) (73% reduction of blood glucose at 50 mg/kg) [197] and azocycloalkane (**71**) [198] are not reported. PPARγ is the proposed receptor for the antidiabetic BM-17.0744 (**72**) which may also improve cardiac power [199]. The indole compounds, such as **73-75**, lower blood glucose by 19% in *db/db* mice (3.2 mg/kg over 14 days), reduce triglycerides, and are reported to inhibit phosphodiesterase type 5 (PDE5) [200]. Others in the series also decrease circulating blood glucose concentrations in *db/db* mice and are either inhibitors of PDE5 or cGMP phosphodiesterase (cGMP-PDE) [201].

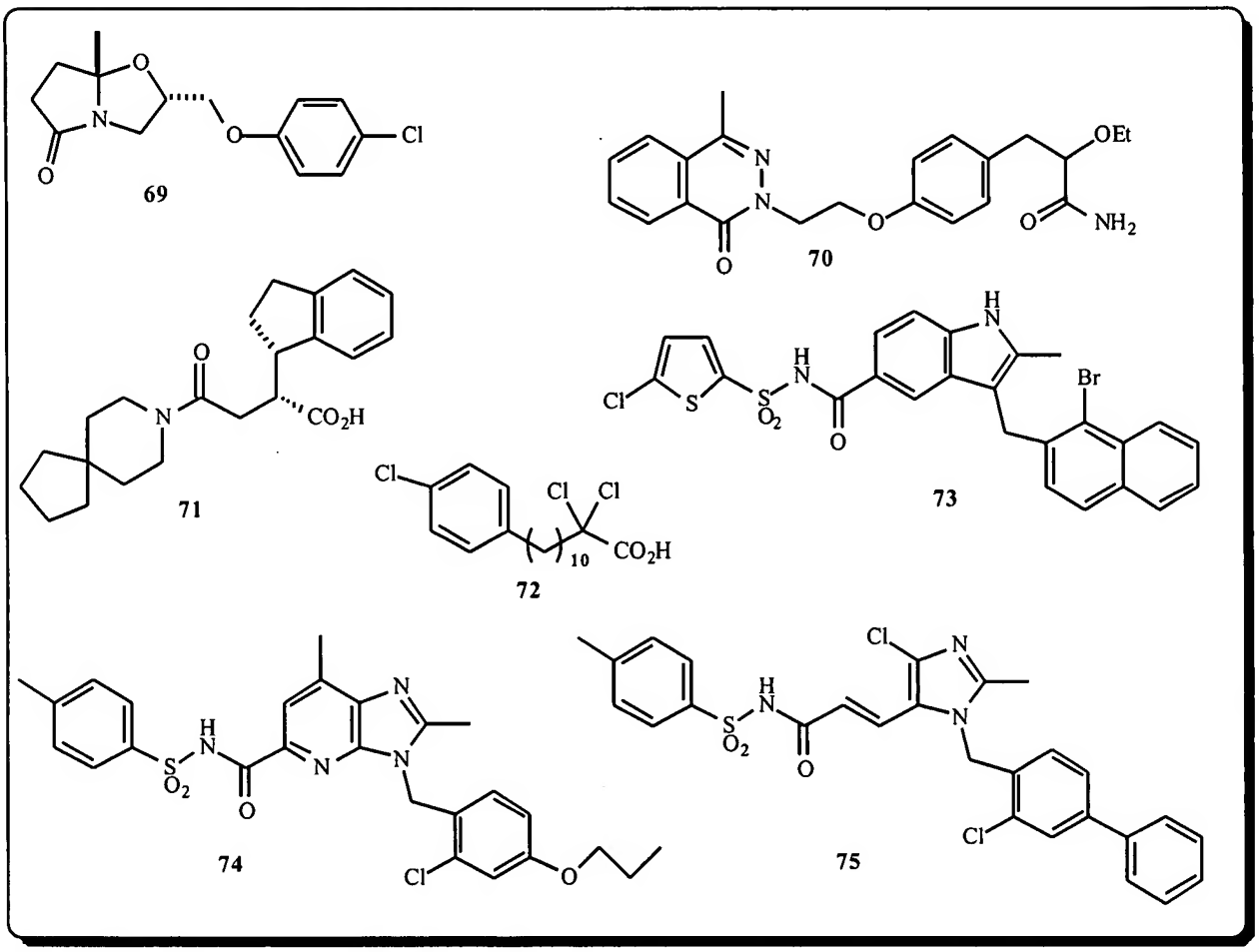
INHIBITORS OF GLUCOSE UPTAKE

Glycosidase Inhibitors

Inhibition of α-glucosidases in the upper gastrointestinal tract limits the degradation of fructose to glucose and delays the breakdown of complex polysaccharides until further along in the small intestine reducing the postprandial glucose peak [202]. Acarbose was the first member of this class and its clinical use is well reviewed [203-205]. The side effects and safety of acarbose have also been examined [206,207]. Voglibose has been evaluated in NIDDM patients and in combination with pioglitazone in GK rats [208,209]. Miglitol in NIDDM subjects has shown a modest 0.3-0.7% reduction of HbA1c and a slight decrease in serum insulin [210]. The prospects for the use of naturally occurring alkaloidal sugar mimics such as polyhydroxylated piperidines, pyrrolidines, and indolizidines as antidiabetics has been reviewed [211].

Inhibition of Gastric Emptying

Delaying the process of gastric emptying moderates postprandial glucose spikes. Several inhibitors based on natural human hormones or analogues are being pursued such as amylin

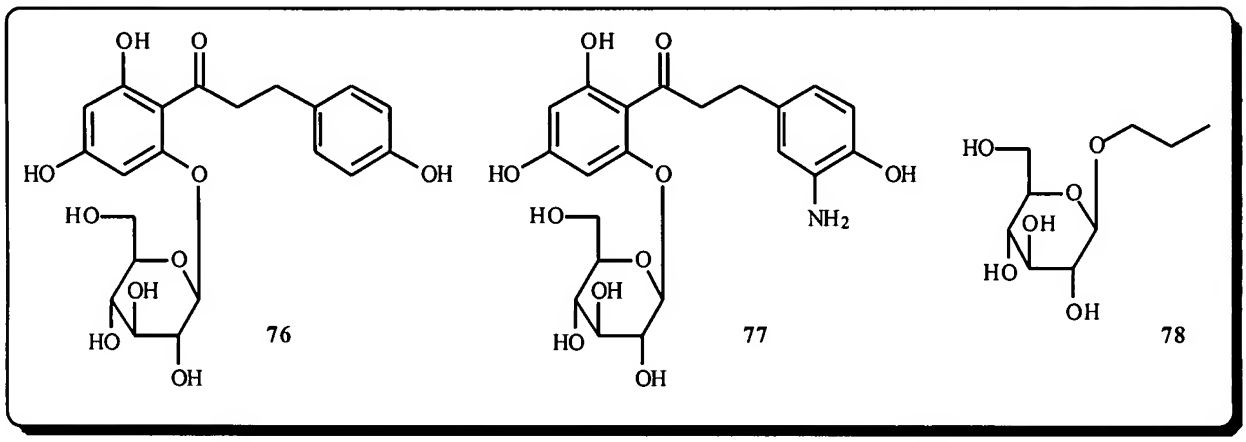


(pramlintide or the synthetic symlin) [212,213], exendin-4 (AC-2993) [214], and cholecystokinin octapeptide (CCK-8) [215].

Inhibition of Na^+-Glucose Cotransporter (SGLT)

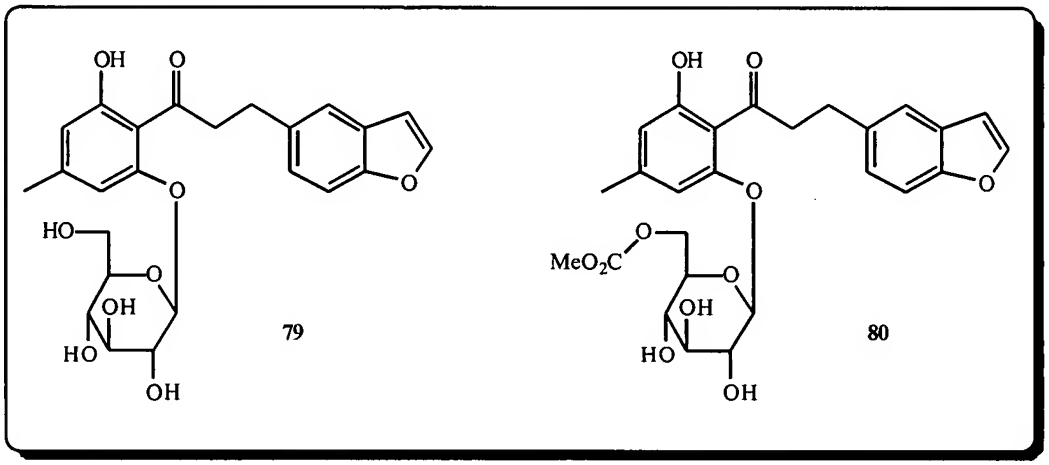
SGLTs represent a novel approach to the treatment of diabetes. While most treatments focus on either improved glucose disposal, controlling hepatic glucose release, or blocking glucose absorption in the gut, inhibitors of SGLTs can block renal glucose reabsorption from urine. Since the underlying toxic effect in either type 1 or type 2 diabetes is hyperglycemia, excreting excess glucose should be a viable method for maintaining blood glucose control in both forms of the disease. There are at least 3 forms of SGLT which have been reported including SGLT1 [216], SGLT2 [217], and SGLT3 (SAAT1-pSGLT2) [218]. Since SGLTs are found in high concentrations in both the intestine and in the kidney, glucose control could be approached by inhibition of glucose absorption through the gut or through renal excretion. Specifically, SGLT1 is found in the epithelial cells of the intestine and kidney, while SGLT2 is found only in renal epithelium cell [219]. SGLT3 is reported to exist in several tissues including intestine, spleen, liver, muscle, and in lesser amounts in the kidney [220]. There is also some differentiation between the SGLTs in the way they mediate glucose reabsorption in conjunction with sodium, SGLT1 acts as a high-affinity low-capacity transporter while SGLT2 and 3 function as low-affinity high-capacity transporters [221]. Thus, SGLT's show a variety of mechanisms to maintain normal glycemia, but mainly show their activity in absorption of glucose through the small intestine and reabsorption of glucose in the early proximal tubule segment.

To date there are only a few published studies exploring this approach to treat hypergylcemia. Historically, phlorizin (**76**) (a natural product



derived from apple trees) has been used to induce glycosuria by inhibiting SGLTs in the kidney, but this compound's low oral bioavailability has restricted its use as a therapy [222]. Phlorizin (**76**) has been shown effective as an inhibitor of renal glucose reabsorption in streptozotocin treated rats and alloxan treated dogs through *iv* and *sc* administration. These studies showed promising results for SGLT inhibitors by restoring normoglycemia and as a consequence improving the glucose sensitivity of pancreatic α-cells [223]. Some structure-activity relationships have been developed based on D-glucose and phlorizin analogues. In a series of analogues based on phlorizin, changes at C-3 gave the following order of potency: amino (**77**), bromo, azido, nitro, and dansyl [224]. While some glucosides inhibit glucose transport generally, n-octyl-D-glucose (**78**) (alpha and beta) inhibits SGLT1 specifically [225]. Early work on orally available 4'-dehydroxyphlorizin derivatives resulted in significant urinary glucose excretion (100 mg/kg po), reduction in blood glucose, and specific inhibition of SLGT's over GLUT-1 [226]. Continued medicinal chemistry on the series lead to modifications to the B-ring of phlorizin. Specifically, small alkyl groups substituted on the 4'-position gave a boost in activity as seen in T-1095A (**79**). To overcome hydrolysis of the compounds by β-glucosidases in the gut, acyl esters of the lead compound were made as prodrugs yielding T-1095 (**80**) which is the most efficacious SGLT inhibitor judging from oral glucose tolerance tests in *db/db* mice [227]. Thus it is possible to produce selective and reversible inhibitors of SGLT which are in effect inactive against the GLUTs, which induce urinary glucose excretion by oral administration, and which suppress hyperglycemia.

One major adverse side effect of some diabetes therapies is hypoglycemia. Inhibitors of SGLT seem to avoid this detraction even under fasted conditions in KK-A^y mice. While the mechanism



of action is inhibition of glucose reabsorption, presumably the SGLTs are not completely inhibited and thus glucose levels are not suppressed below normal. Under normoglycemic conditions, glucose in the glomerular filtrate is below the maximal absorptive capacity of the proximal tubules and glucose is completely reabsorbed. In diabetic models where the glucose concentrations exceed the renal maximal absorptive capacity, urinary glucose excretion increases linearly with increases in blood glucose concentrations. Thus partial inhibition of SGLTs is effective in lowering hyperglycemia, but not significantly below normal levels [227].

An SGLT inhibitor could also block glucose uptake in the gut acting in a similar manner as acarbose which might lead to gastrointestinal distress as observed with α-glucosidase inhibitors. Indeed phlorizin (**76**) which is poorly absorbed has a post-prandial effect on glucose absorption in the intestine. Alternatively, T-1095 (**80**) is highly bioavailable and exerts its effect renally [228]. Increased glucose excretion raises concerns over possible polyuria and dehydration. However, 4-6 week studies with T-1095 (**80**) showed no renal toxicity, and urinary glucose excretion and urine volume decreased over the treatment period with correction of hyperglycemia. The decrease in urinary glucose is explained by reduction of circulating blood glucose concentrations, increased insulin sensitivity, normalizing β cell function, and improved glucose disposal [229]. Further preclinical and clinical studies will help determine if correction of hyperglycemia by inhibition of SGLTs can maintain normal glucose metabolism in diabetic patients.

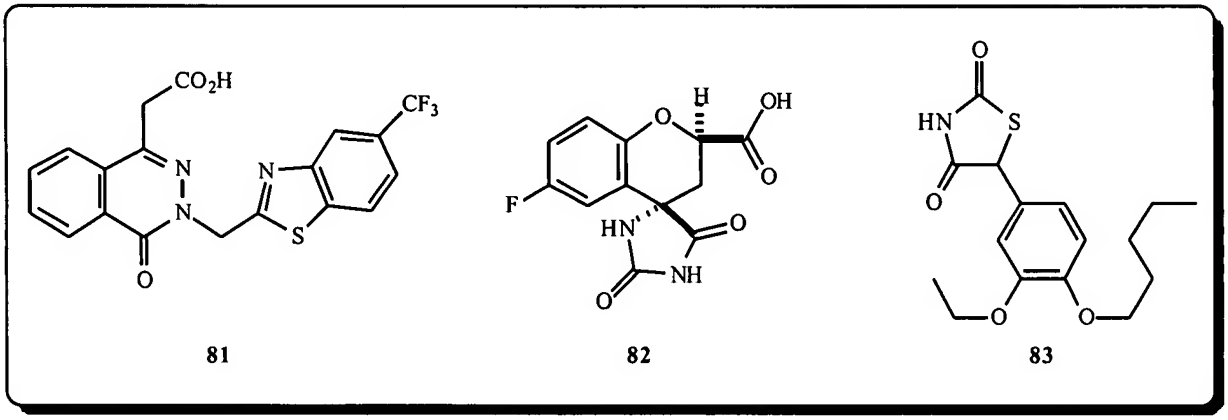
Miscellaneous Antidiabetics

Vanadium is implicated in modification of phosphorylation/dephosphorylation steps in the insulin cascade which result in hyperglycemic control [230]. The potent dopamine D2 receptor agonist bromocriptine (Ergoset) has been shown to improve glycemic control and hyperlipidemia in humans [231]. Bromocriptine and SKF 38393 (a dopamine D1 receptor agonist) act synergistically to reduce blood glucose in *ob/ob* mice by altering metabolic and endocrine pathways [232].

AGENTS FOR THE TREATMENT OF DIABETIC COMPLICATIONS

Aldose Reductase Inhibitors (ARIs)

Of great concern in the long term treatment of diabetes is the development of associated complications especially neuropathy, nephropathy, retinopathy, and cataract [233,234]. Although the mechanism of action is not clear, the therapeutic effect of ARIs is believed to be connected to the products of cytosolic glucose metabolism, such as sorbitol which is important for renal osmotic control. Inhibition of sorbitol dehydrogenase, which increases the concentration of sorbitol in tissue, has a deleterious effect on neuropathy and cataract formation, and helps prove the utility of aldose reductase inhibitors [235,236]. Zopolrestat (**81**) is the first drug reported to reverse preexisting renal hyperperfusion as well as prevent the condition in diabetic rats [237]. 3-Deoxyglucosone (3-DG), which reacts with protein amino groups giving advanced glycation products (AGEs), has been linked to diabetic and uremic complications through AR. The use of ARIs reduced the amount of erythrocyte 3-DG and AGEs which suggests a new mechanistic role for aldose reductase [238]. The crystal structure of human AR with its cofactor, NADP, has been solved [239]. Topical application of 1-3% Tolrestat (**82**) was shown to prevent cataract in rats [240]. CT-112 (**83**), which is being developed as a topical treatment for keratopathy, and AL-1576 stopped loss of corneal sensitivity in galactose-fed rats [241]. Other compounds being evaluated for retinopathy include M79175 [242], a tetrazol (TAT) [243], the benzothiazol GP-1447 [244], SNK-860 [245], and tetrahyroquinazoline [246]. The carboxylic acid compound IDD-676 is effective in preventing nerve dysfunction in streptozotocin-diabetic rats [247]. Several other ARIs are being pursued as treatments for diabetes related complications including Minalrestat (ARI-509) [248], AS-3201 [249], and indolealkanoic acids [250].



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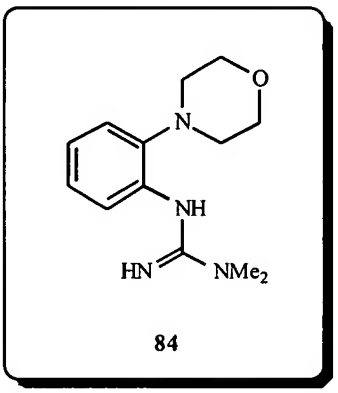
Polyoldiphenyl compounds have also been recently identified as potent ARI's [251]. The x-ray crystal structure of human AR has been solved as the co-crystal with ARI fidarestat [252]. After many years of clinical trials, the utility of ARIs has not been conclusively proven. While increasingly potent inhibitors of ARI have been discovered, the number of patents in the area has decreased over time possibly in relation to the lack of commercial success derived from the target. Research activity has also been focused on the next enzyme downstream in the polyol pathway, sorbitol dehydrogenase (SDH). Unfortunately, SDH does not seem to be a safe and effective target [253]. For treatment of diabetic neuropathy, the vascular activity of C-peptide and endothelin-1 are being examined as novel avenues of therapy.

Advanced Glycation End-Products (AGE)

Highly reactive glycating compounds like the dicarbonyl-containing methylglyoxal and glyoxal form covalent adducts with proteins resulting in AGEs. A wide variety of AGEs accumulate in tissues and in circulating plasma in relation to aging, diabetes, and kidney failure. AGEs have been associated with vascular complications in diabetics and the progression of atherosclerosis caused for example through crosslinking of arterial wall proteins. AGEs interact with specific receptors called RAGE which have been linked to induction of mRNAs and several proteins including fibronectin and collagen type I [254]. The biology, disease pathology, and some treatments based on inhibition of AGEs has been recently review [255]. Nucleophilic guanidines like metformin and BTS-67-682 (**84**) are thought to inhibit AGE formation by reacting with glycating compounds before they can react with proteins. When co-incubated, metformin inhibits formation of glycation products of albumin by about 40% over controls [256]. In assays with BSA or lysine and glucose, BTS-67-582 (**84**) at 200mM inhibited AGE formation by about 70% [257]. Aminoguanidine has been shown to react with α-oxoaldehydes *in vitro* and *in vivo* to form 3-amino-1,2,4-triazines. Thus, aminoguanidine should be effective for inhibiting the accumulation of AGEs. The kinetics of the reaction between aminoguanidine and α-oxoaldehydes (glyoxal, methylglyoxal, and 3-deoxyglucosone) were studied under physiological conditions, and the results indicate that aminoguanidine should be active enough *in vivo* to prevent gylcation of proteins [258]. Unfortunately, the efficacy of aminoguanidine and related compounds is limited by their high rate of renal clearence. Aminoguanidine was used to assess intracellular AGE formation and the progression of NIDDM in



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diabetic mice [259]. While studies in rodent models showed promising results, a three year study of the effects of aminoguanidine in Type I diabetic baboons provided no improvement in nerve dysfunction and no effect on glucose control [260].

Unlike the mechanism of aminoguanidine, the inhibitory effect of dilazep, an antiplatelet agent, on AGEs may be attributed to its ability to scavenge free-radicals [261]. Other compounds like N-phenacylthiazolium bromide, are thought to act on pre-formed AGEs by breaking the protein cross-links. The effect of N-phenacylthiazolium bromide and a novel AGE inhibitor, NNC39-0028 (2,3-diaminophenazine), were examined in streptozotocin-treated female Wistar rats. While there was a significant therapeutic effect found in the NNC39-0028 treated animals, there was no effect observed in the N-phenacylthiazolium bromide treated rats by measurement of clearance of 125I-albumin in the eye [262]. In a separate study, N-phenacylthiazolium bromide was found to arrest increases in vascular AGE accumulation by a direct determination of vascular AGE products [263]. With these recent mixed results in animal models, only further animal studies focusing on clinical endpoints will determine if AGE cross-link breakers are effective for the treatment of vascular dysfunction. By analogy to AGE inhibitors, a novel direction for therapy may come for decreasing the enzyme activity of fucosyl and sialyltransferase which would change the carbohydrate content of some glycoproteins and may have an effect on retinopathy [264].

Angiotensin II type 1 (AT1) Receptor

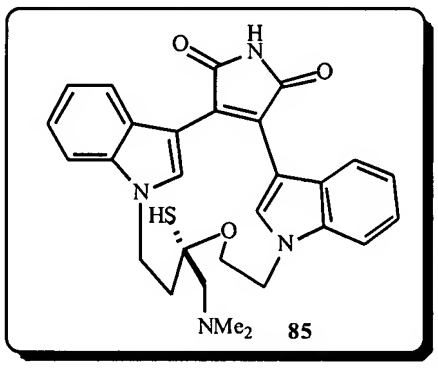
Six highly selective AT1 inhibitors (losartan, valsartan, irbesartan, candesartan, telmisartan, and eprosartan) are available for treatment of hypertension. Clinical trials are underway to evaluate their benefits for NIDDM related nephropathy [265]. The renoprotective performance of AT1 inhibitors has been reviewed [266]. Unlike ACE inhibitors, AT1 antagonists do not increase bradykinin levels. The renoprotective effects of AT1 inhibitors have also been linked to increased mesangial matrix accumulation and TGF-β1 secretion in mesangial cells [267]. The nephroprotective action may also be related to lipid peroxidation mechanisms [268].

Proinsulin C-peptide

Proinsulin C-peptide plays an important role in proper protein folding in the biosynthesis of insulin. C-peptide is released in equimolar amounts with insulin, but has generally been considered physiologically inert. Research has recently revealed that C-peptide has improved renal function, increased glucose utilization, and increased skin and muscle blood flow in type 1 diabetic patients. The effects may be associated with stimulation of Na+, K+ ATPase, and endothelial nitric oxide synthase (eNOS) [269].

Protein Kinase C (PKC)

Typically a reduction in the activity of PKC is thought to contribute to insulin insensitivity. Recently however, a PKC-β isozyme selective inhibitor LY333531 (**85**) was shown to have beneficial effects on the prevention of diabetic nerve dysfunction, which may be related to PKC's action on the endoneurial micro-vasculature [270]. The isozymes of PKC may also play a part in nephropathy. The research into the glucose enhanced activity of PKC in mesangial cells has shown that the polyol pathway plays a role in PKC isozyme content and cellular location [271].



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EMERGING TARGETS FOR TREATMENT OF DIABETES AND CONCLUSIONS

As the population of diabetics increases, the need for pharmaceutical control of hyperglycemia

is obvious. Of equal importance to glycemic control is the regulation of the peripheral metabolic side effects of hyperglycemia. With a multi-pronged therapeutic approach to diabetes, a patients' quality of life and general health may be maintained for a much longer period of time. Also with an increasing rate of diagnoses, the value of new therapies will grow. The potential market for new drugs, the expanding diabetic population, and pressure from patients for new drugs will continue to spur novel and innovative research into all facets of diabetes therapy. The mainstay therapies, which have been investigated thoroughly through the years, have set the stage for the next generation of targeted drug development programs. The recent emergence of new technologies, the discovery of highly selective binding molecules, and a modern understanding of diabetic pathology has prompted creative research into a wide variety of therapies for glucose control and diabetic complications. The next hurdles facing the drug discovery community are developing potent once-a-day treatments to maintain patient compliance, developing non-injectable alternatives to insulin, and developing combination therapies that work best for profiled subsets of diabetics. The use of current combination therapies has been reviewed [272].

Other than some of the highlighted targets currently being pursued in pharmaceutical companies such as PTP-1B, DPP-IV, and GSK3, a few new areas are starting to attract attention. For example, PPARα agonists for treatment of diabetic complications such as obesity, dyslipidemia, and atherosclerosis. For the control of obesity, the hormone leptin is thought to have a role in mediating food intake and energy expenditure [273]. Lipase inhibitors, such as Orlistat, act favorably in obese type II diabetics to control weight and lipoprotein cholesterol levels [274]. The quest for the genetic basis of the disease will likely yield many more new, as yet unknown therapeutic targets as more genomic information is interpreted. Gene therapy has been proposed to prevent destruction or restore β-cell function specifically in type I diabetics [275]. Gene therapy approaches employing direct delivery of the insulin gene, either to non-secreting or secreting cells, or through the development of ex-vivo approaches using genetically modified cells, though still in the very early stages, are also under investigation and hold considerable promise for diabetics. If fact with the considerable advances in high through-put screening, elucidation of protein interactions and function, and great strides unraveling the genetic roots of diabetes, the stage is set for the discovery of many new genomic-based targets and novel efficacious therapies.

REFERENCES

[1] "Prevention of Diabetes Mellitus", *World Health Organization Technical Report Series* **1994**, No. 844.

[2] "Diabetes Mellitus", *World Health Organization Fact Sheet* **1999**, No. 138.

[3] "Diabetes Mellitus", American Diabetes Association - General Fact Sheet, **1999**.

[4] "Diabetes Mellitus", *World Health Organization Fact Sheet* **1999**, No. 236.

[5] American Diabetes Association, *Diabetes Care* **1999**, *22* (Suppl. 1), S27.

[6] Turner, R. C.; Cull, C. A.; Frighi, V.; Holman, R. R. *J. Am. Med. Assoc.* **1999**, *281*, 2005.

[7] UK Prospective Diabetes Group, *Lancet* **1998**, *352*, 837.

[8] Matthews, D. R. *Exp. Clin. Endocrinol. Diabetes* **1999**, *107*(Suppl. 2), S34.

[9] Heinemann, L. *J. Diabetes Compl.* **1999**, *13*, 105.

[10] Brange, J.; Volund, A. *Adv. Drug Delivery Rev.* **1999**, *35*, 307.

[11] Chetty, D. J.; Chen, Y. W. *Crit. Rev. Ther. Drug Carrier Syst.* **1999**, *15*, 629.

[12] Zhang, B.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M.; Pelaez, F.; Ruby, C.; Kendall, R.; Mao, X.; Griffin, P.; Calaycay, J.; Zierath, J.; Heck, A.; Smith, R.; Moller, D. *Science* **1999**, *284*, 974.

[13] Liu, K.; Xu, L.; Szalkowski, D.; Li, Z.; Ding, V.; Kwei, G.; Huskey, S.; Moller, D.; Heck, J.; Zhang, B.; Jones, A. *J. Med. Chem.* **2000**, *43*, 3487.

[14] Liu, K.; Jones, A. B.; Wood, H. B.; Zhang, B. WO 9951225, **1999**.

[15] Burge, M. R.; Sood, V.; Sobhy, T. A.; Rassam, A. G.; Schade, D. S. *Diabetes, Obes. Metab.* **1999**, *1*, 199.

[16] Bataille, D.; Heron, L.; Virsolvy, A.; Peyrollier, K.; LeCam, A.; Blache, P. *Cell. Mol. Life Sci.* **2000**, *56*, 78.

[17] Burge, M. R.; Sood, V.; Sobhy, T. A.; Rassam, A. G.; Schade, D. S. *Diabetes, Obes. Metab.* **1999**, *1*, 199.

[18] Malaisse, W. J. *Exp. Opin. Clin. Endocrinol. Diabetes* **1999**, *107*(Suppl. 4), S140.

[19] Kikuchi, M. *Sogo Rinsho* **1996**, *45*, 2765.

[20] Akita, H.; Imamura, Y.; Sanui, K.; Kurashima, K.; Seri, K. WO 99/48864, **1999**.

[21] Shinkai, H.; Ozeki, H.; Motomura, T.; Ohta, T.; Furakawa, N.; Uchida, I. *J. Med. Chem.* **1998**, *41*, 5420.

[22] (a) Drucker, D. J. *Diabetes* **1998**, *47*, 159.

[23] Gallwitz, B.; Ropeter, T.; Morys-Wortmann, C.; Mentlein, R.; Schmidt, W. E. *Regul. Pep.* **2000**, *86*, 103.

[24] Xu, G.; Stoffers, D. A.; Habener, J. F.; Bonner-Weir, S. *Diabetes* **1999**, *48*, 2270.

[25] Burcelin, R.; Rolland, E.; Dolci, W.; Germane, S.; Carrel, V.; Thorens, B. *Ann. NY Acad. Sci.* **1999**, *875*, 277.

[26] Augustyns, K.; Bal, G.; Thonus, G.; Belyaev, A.; Zhang, X. M.; Haemers, A. *Curr. Med. Chem.* **1999**, *6*, 311.

[27] Hughes, T. E.; Mone, M.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. *Biochemistry* **1999**, *38*, 11597.

[28] LeBihan, G.; Rondu, F.; Pele-Tounian, A.; Wang, X.; Lidy, S.; Touboul, E.; Lamouri, A.; Dive, G.; Huet, J.; Pfeiffer, B.; Renard, P.; Godfroid, J. J. *J. Med. Chem.* **1999**, *42*, 1587.

[29] Kramer, W. *Exp. Clin. Endocrinol. Diabetes* **1999**, *107*(Suppl. 2), S52.

[30] Stacpoole, P.; Greene, Y. *Diabetes Care* **1992**, *15*, 785.

[31] Bressler, R.; Johnson, D. *Diabetes Care* **1992**, *15*, 792.

[32] Randle, P.; Kerby, A.; Espinal, J. *Diabetes Metab. Rev.* **1988**, *4*, 623.

[33] Thiebaud, D.; DeFronzo, R.; Jacot, E.; Golay, A.; Acheson, K.; Maeder, E.; Jequier, E.; Felber, J. *Metab.,Clin. Exp.* **1982**, *31*(11), 1128.

[34] Garg, A.; Grundy, S. *JAMA* **1990**, *264*, 723.

[35] Fulcher, G.; Catalano, C.; Walker, M.; Farrer, M.; Thow, J.; Whately-Smith, C.; Alberti, K. *Diabet. Med.* **1992**, *9*(10), 908.

[36] Giugliano, D. Int. Congr. Ser. **1999**, *231*, 1177 (Insulin Resistance, Metabolic Diseases and Diabetic Complications).

[37] Wiernsparger, N. F. *Diabetes Metab.* **1999**, *25*, 110.

[38] UK Prospective Diabetes Group *Lancet* **1998**, *352*, 837.

[39] Livingston, J. N.; Schoen, W. R. *Ann. Rep. Med. Chem.* **1999**, *34*, 189.

[40] Cascieri, M. A.; Koch, G. E.; Ber, E.; Sadowski, S. J.; Louizides, D.; deLaszlo, S. E.; Hacker, C.; Hagmann, W. K.; MacCoss, M.; Chicchi, G. G.; Vicario, P. P. *J. Biol. Chem.* **1999**, *274*, 8694.

[41] deLaszlo, S. E.; Hacker, C.; Li, B.; Kim, D.; MacCoss, M.; Mantlo, N.; Pivnichny, J.; Colwell, L.; Kock, G. E.; Cascieri, M. A.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 641.

[42] Hoover, D. J.; Lefkowitz-Snow, S.; Burgess-Henry, J. L.; Martin, W. H.; Armento, S. J.; Stock, I. A.; McPherson,R. K.; Genereux, P.; Gibbs, E. M.; Treadway, J. L. *J. Med. Chem.* **1998**, *41*, 2934.

[43] Martin, W. H.; Hoover, D. J.; Armento, S. J.; Stock, I. A.; McPherson, R. K.; Danley, D. E.; Stevenson, R. W.; Barrett, E. J.; Treadway, J. L. *Proc. Nat. Acad. Sci.* **1998**, *95*, 1776.

[44] Treadway, J. L. *Diabetes* **1998**, *47* (Suppl. 1), Abst 1115.

[45] Aicher, T. D.; Anderson, R. C.; Bebernitz, G. R.; Coppola, G. M.; Jewell, C. F.; Knorr, D. C.; Liu, C.; Sperbeck, D. M.; Brand, L. J.; Stohschein, R. J.; Gao, J.; Vinluan, C. C.; Shetty, S. S.; Mann, W. R. *J. Med. Chem.* **1999**, *42*, 2741.

[46] Islam, A. *Diabetes* **1999**, *48* (Suppl. 1), Abst. 2012.

[47] Butlin, R. J.; Burrows, J. N. WO 9962873, **1999**.

[48] Aicher, T. D.; Anderson, R. C.; Gao, J.; Shetty, S.; Coppola, G. M.; Islam, A.; Walter, R. E.; Mann W. R. *J. Med. Chem.* **2000**, *43*, 236.

[49] Mjalli, A. M. M.; Mason, J. C.; Arienti, K. L.; Short, K. M.; Kimmich, R. D. A.; Jones, T. K. WO 9947549, **1999**.

[50] Parker, J. C.; VanVolkenburg, M. A.; Maria, A.; Levy, C. B.; Martin, W. H.; Burk, S. H.; Kwon, Y.; Giragossian, C.; Gant, T. G.; Carpino, P. A.; McPherson, R. K.; Vestergaard, P.; Treadway, J. L. *Diabetes* **1998**, *47*, 1630.

[51] Auwerx, J. *Diabetologia* **1999**, *42*, 1033.

[52] Gelman, L.; Fruchart, J.; Auwerx, J. *Cell. Mol. Life Sci.* **1999**, *55*, 932.

[53] Kersten, S.; Desvergne, B.; Wahli, W. *Nature* **2000**, *405*, 421.

[54] Rocchi, S.; Auwerx, J. *Ann. Med.* **1999**, *31*, 342.

[55] Kolaczynski, J.; Caro, J. *J. Basic Clin. Physiol. Pharmacol.* **1998**, *9*, 281.

[56] Lowell, B. *Cell* **1999**, *99*, 239.

[57] Ricote, M.; Huang, J.; Welch, J.; Glass, C. *J. Leukoc. Biol.* **1999**, *66*, 733.

[58] Barak, Y.; Nelson, M.; Ong, E.; Jones, Y.; Ruiz-Lozano, P.; Chien, K.; Koder, A.; Evans, R. *Mol. Cell.* **1999**, *4*, 585.

[59] Kubota, N.; Terauchi, Y.; Miki, H.; Tamemoto, H.; Yamauchi, T.; Komeda, K. *Mol. Cell* **1999**, *4*, 597.

[60] Fruchart, J.; Duriez, P.; Staels, B. *Curr. Opin. Lipidol.* **1999**, *10*, 245.

[61] Vanden Heuvel, J. *Toxicol. Sci.* **1999**, *47*, 8.

[62] (a) Wu, Z.; Xie, Y.; Morrison, R.; Bucher, N.; Farner, S. *J. Clin. Invest.* **1998**, *101*, 22. (b) Young, P.; Cawthorne, M.; Coyle, P.; Holder, J.; Holman, G.; Kozka, I.; Kirkham, D.; Lister, C. Smith, S. *Diabetes* **1995**, *44*, 1087.

[63] Hollenberg, A.; Susulic, V.; Madura, J.; Zhang, B.; Moller, D.; Tontonoz, P.; Sarraf, P.; Spiegelman, B.; Lowell, B. *J. Biol. Chem.* **1997**, *272*(8), 5283.

[64] Kubota, N.; Terauchi, Y.; Miki, H.; Tamemoto, H.; Yamauchi, T.; Komeda, K.; Satoh, S.; Nakano, R.; Ishii, C.; Sugiyama, T.; Eto, K.; Tsubamoto, Y.; Okuno, A.; Murakami, K.; Sekihara, H.; Hasegawa, G.; Naito, M.; Toyoshima, Y.; Tanaka, S.; Shiota, K.; Kitamura, T.; Fujita, T.; Ezaki, O.; Aizawa, S.; Nagai, R.; Tobe, K.; Kimura, S.; Kadowaki, T. *Mol. Cell* **1999**, *4*(4), 597.

[65] (a) Camirand, A.; Marie, V.; Rabelo, R.; Silva, J. *Endocrinology* **1998**, *139*, 428. (b) Aubert, J.; Champigny, O.; Saint-Marc, P.; Negrel, R. : Collins, S.; Ricquier, D.; Ailhaud, G. Biochem. Biophys. Res. Commun. **1997**, *238*, 606.

[66] Kagawa, Y.; Cha, S.; Hasegawa, K.; Hamamoto, T.; Endo, H. *Biochem. Biophys. Res. Commun.* **1999**, *266*(3), 662.

[67] Puigserver, P.; Adelmant, G.; Wu, Z.; Fan, M.; Xu, J.; O'Malley, B.; Spiegelman, B. *Science* **1999**, *286*(5443), 1368.

[68] Vega, R.; Huss, J.; Kelly, D. *Mol. Cell. Biol.* **2000**, *20*(5), 1868.

[69] (a) Baumann, C.; Chokshi, N.; Saltiell, A.; Ribon, V. *J. Biol. Chem.* **2000**, *275*(13), 9131. (b) Ribon, V.; Johnson, J.; Camp, H.; Saltiel, A. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*(25), 14751.

[70] Wilson, T.; Brown, P.; Sternbach, D.; Henke, B. *J. Med. Chem.* **2000**, *43*, 527.

[71] Goldstein, B. *Diabetes Technol. Ther.* **1999**, *1*, 267.

[72] Levin, K.; Beck-Nielsen, H. *Int. Congr. Ser.* **1999**, *1177*, 243.

[73] Reginato, M.; Lazar, M. *Trends Endocrinol. Metab.* **1999**, *10*, 9.

[74] Komers, R.; Vrana, A. *Physiol. Res. (Prague)* **1998**, *47*, 215.

[75] Day, C. *Diabet. Med.* **1999**, *16*, 179.

[76] Barroso, I.; Gumell, M.; Crowley, S.; Agostinil, M.; Schwabe, J.; Soos, M.; Maslen, G.; Williams, T.; Lewis, H.; Schafer, A.; Chatterlee, V.; O'Rahilly, S. *Nature* **1999**, *402*, 880.

[77] *Scrip* **1997**, *2204*, 17.

[78] *Scrip* **1997**, *2272*, 23.

[79] Plosker, G.; Faulds, D. *Drugs* **1999**, *57*, 409.

[80] Johnson, M.; Campbell, L.; Campbell, R. *Ann. Pharmacother.* **1998**, *32*, 337.

[81] Subramaniam, S. *Clin. Exp. Hypertens.* **1999**, *21*, 121.

[82] Wagenaar, L.; Kuck, E.; Hoekstra, J. *Neth. J. Med.* **1999**, *55*, 4.

[83] Jones, S.; Moore, L.; Shenk, J.; Wisely, G.; Hamilton, G.; McKee, D.; Tomkinson, N.; LeCluyse, E.; Lambert, M.; Willson, T. Kliewer, S.; Moore, J. *Mol. Endocrinol.* **2000**, *14*, 27.

[84] F. D. A., Talk Paper, June, **1999**.

[85] Buysschaert, M.; Bobbioni, E.; Starkie, M.; Frith, L. *Diabet. Med.* **1999**, *16*, 147.

[86] Inzucchi, S.; Maggs, D.; Spollett, G.; Page, S.; Rife, F.; Walton, V.; Shulman, G.; *N. Engl. J. Med.* **1998**, *338*, 867.

[87] Matsumoto, K.; Miyake, S.; Yano, M.; Ueki, Y.; Tominaga, Y. *Metab.,Clin. Exp.* **1999**, *48*, 1.

[88] Umland, E.; Romanelli, A. *Ann. Pharmacother.* **1999**, *33*, 229.

[89] The Pink Sheet, F-D-C Reports, Inc., July 19 **1999**.

[90] Yamasaki, Y.; Kawamori, R.; Wasada, T.; Sato, A.; Omori, Y.; Eguchi, H.; Tominaga, M.; Sasaki, H.; Ikeda, M.; Kubota, M.; Ishida, Y.; Hozumi, T.; Baba, S.; Uehara, M.; Shichiri, M.; Kaneko, T. *J. Exp. Med.* **1997**, *183*, 173.

[91] Sohda, T.; Ikeda, H.; Meguro, K. *J. Chem. Pharm. Bull.* **1995**, *43*, 2168.

[92] *Scrip* **1999**, *2345*, 19.

[93] SmithKline Beecham, *Press Release*, June 21 **1999**.

[94] Balfour, J.; Plosker, G. *Drugs* **1999**, *57*, 921.

[95] Freid, J.; Everitt, D; Boscia, J. *Ann. Intern. Med.* **2000**, *132*, 164.

[96] Al-Salman, J.; Arjomand, H; Kemp, D.; Mittal, M. *Ann. Intern. Med.* **2000**, *132*, 121. ibid **2000**, *133*(3), 237.

[97] Forman, L.; Simmons, D.; Diamond, R.; *Ann. Intern. Med.* **2000**, *132*, 118.

[98] Adams, M.; Raman, P.; Judd, R.; *Biochem. Pharmacol.* **1998**, *55*, 1915.

[99] Stevenson, R.; Kreutter, D.; Andrews, K.; Genereux, P.; Gibbs, E.; *Diabetes* **1998**, *47*, 179.

[100] Jacinto, S. *Diabetologia* **1999**, *42*(Suppl. 1), Abst. 675.

[101] Sohda, T.; Odaka, H.; Momose, Y. WO 9605186, **1996**.

[102] *Scrip* **1998**, *2335*, 28.

[103] Willson, T.; Cobb, J.; Cowan, D.; Wiethe, R.; Correa, I.; Prakash, S.; Beck, K.; Moore, L.; Kliewer, S.; Lehmann, J. *J. Med. Chem.* **1996**, *39*, 665.

[104] Pickavance, L.; Widdowson, P.; King, P.; Ishii, S.; Tanaka, H.; Williams, G. *Br. J. Pharmacol.* **1998**, *125*, 767.

[105] Upton, R.; Widdowson, P.; Ishii, S.; Tanaka, H.; Williams, G. *Br. J. Pharmacol.* **1998**, *125*, 1708.

[106] Wrobel, E.; Li, Z; Dietrich, A. WO 9524400, **1995**.

[107] Ohara, Y.; Suzuki, M.; Ohdoi, K.; Miyachi, N.; Kato, K.; Kobayashi, T.; Shikada, K.; Kitahara, M.; Naito, T. WO 9626207, **1996**.

[108] Wrobel, J.; Li, Z.; Dietrich, A.; McCaleb, M.; Mihan, B.; Sredy, J.; Sullivan, D. *J. Med. Chem.* **1998**, *41*, 1084.

[109] Arakawa, K.; Ishihara, T.; Aoto, M.; Inamasu, M.; Saito, A.; Ikezawa, K. *Br. J. Pharmacol.* **1998**, *125*, 429.

[110] Murakami, K.; Tsunoda, M.; Ide, T.; Ohashi, M.; Mochizuki, T. *Metab. Clin. Exp.* **1999**, *48*, 1450.

[111] (a) Nomura, M.; Kinoshita, S.; Satoh, H.; Maeda, T.; Murakami, K.; Tsunoda, M.; Miyachi, H.; Awano, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 533. (b) Murakami, K.; Tobe, K.; Ide, T.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. *Diabetes* **1998**, *47*, 1841.

[112] Lohray, B.; Bhushan, V.; Bheema Rao, P.; Madhavan, G.; Murali, N.; Rao, K.; Reddy, K.; Rajesh, B.; Reddy, P.; Chakrabarti, R.; Rajagopalan, R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 785.

[113] Lohray, B.; Bhushan, V.; Bheema Rao, P.; Madhavan, G.; Murali, N.; Narasimha Rao, K.; AnanthaReddy, K.; Rajesh, B.; Ganapathy Reddy, P.; Chakrabarti, R.; Vikramadithyan, R.; Rajagopalan, R.; Rao, N.; Mamidi, H.; Jajoo, K.; Swaminathan, S. *J. Med. Chem.* **1998**, *41*, 1619.

[114] Lohray, B.; Bhushan, V.; Reddy, A.; Rao, B.; Reddy, N.; Harikishore, P.; Haritha, N.; Vikramadityan, R.; Chakrabarti, R.; Rajagopalan, R.; Katneni, K. *J. Med. Chem.* **1999**, *42*, 2569.

[115] Willson, T. Book of Abstracts, 216th ACS National Meeting, Boston, **1998**, Aug., MEDI-381.

[116] Brown, K.; Henke, B.; Blanchard, S.; Cobb, J.; Mook, R.; Kaldor, I.; Kliewer, S.; Lehmann, J.; Lenhard, J.; Harrington, W.; Novak, P.; Faison, W.; Binz, J.; Hashim, M.; Oliver, W.; Brown, H.; Parks, D.; Plunket, K.; Tong, W.; Menius, J.; Adkison, K.; Noble, S.; Willson, T. *Diabetes* **1999**, *48*, 1415.

[117] Willson, T.; Mook, R.; Kaldor, I.; Henke, B.; Deaton, D.; Collins, J.; Cobb, J. WO 9731907, **1997**.

[118] Glaxo Wellcome plc., UK, *Company Pipeline*, **1999**, Jan.

[119] Maegawa, H.; Obata, T.; Shibata, T.; Fujita, T.; Ugi, S.; Morino, K.; Nishio, Y.; Kojima, H.; Hidaka, H.; Haneda, M.; Yasuda, H.; Kikkawa, R.; Kashigawi, A. *Diabetologia* **1999**, *42*, 151.

[120] Suzuki, T.; Niigata, K.; Takahashi, T.; Maruyama, T.; Onda, K.; Noshiro, O.; Isomura, Y.; Book of Abstracts, 215th ACS National Meeting, Dallas, **1998**, March, MEDI-044.

[121] Shinkai, H.; Onogi, S.; Tanaka, M.; Shibata, T.; Iwao, M.; Wakitani, K.; Uchida, I. *J. Med. Chem.* **1998**, *41*, 1927.

[122] Shibata, T.; Matsui, K.; Nagao, K.; Shinkai, H.; Yonemori, F.; Wakitani, K. *Eur. J. Pharmacol.* **1999**, *364*, 211.

[123] Henke, B.; Blanchard, S.; Brackeen, M.; Brown, K.; Cobb, J.; Collins, J.; Harrington, W.; Hashim, M.; Hull-Ryde, E.; Kaldor, I.; Kliewer, S.; Lake, D.; Leesnitzer, L.; Lehmann, J.; Lenhard, J.; Orband-Miller, L.; Mook, R.; Noble, S.; Oliver, W.; Parks, D.; Plunkett, K.; Szewczyk, J.; Willson, T. *J. Med. Chem.* **1998**, *41*, 5020.

[124] Collins, J.; Blanchard, S.; Boswell, G.; Charifson, P.; Cobb, J.; Henke, B.; Hull-Ryde, E.; Kazmierski, W.; Lake, D.; Leesnitzer, L.; Lehmann, J.; Lenhard, J.; Orband-Miller, L.; Gray-Nunez, Y.; Parks, D.; Plunkett, K.; Tong, W. *J. Med. Chem.* **1998**, *41*, 5037.

[125] Parks, D.; Tomkinson, N.; Villeneuve, M.; Blanchard, S.; Willson, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3657.

[126] Haigh, D.; Allen, G.; Birrell, H.; Buckle, D.; Cantello, B.; Eggleston, D.; Haltiwanger, R.; Holder, J.; Lister, C.; Pinto, I.; Rami, H.; Sime, J.; Smith, S.; Sweeney, J. *Bioorg. Med. Chem.* **1999**, *7*, 821.

[127] Young, P.; Buckle, D.; Cantello, B.; Chapman, H.; Clapham, J.; Coyle, P.; Haigh, D.; Hindley, R.; Holder, J.; Kallender, H.; Latter, A.; Lawrie, K.; Mossakowska, D.; Murphy, G.; Roxbee Cox, L.; Smith, S. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 751.

[128] Doebber, T.; Berger, J.; Berger, G.; Leibowitz, M.; Moller, D.; Olson, J.; Patchett, A.; Toupence, R. WO 9727847, **1997**.

[129] Santini, C.; Acton, J.; Adams, A.; Aster, S.; Berger, G.; Dadiz, A.; Graham, D.; Gratale, D.; Han, W.; Jones, A.; Patel, G.; Sidler, K.; Sahoo, S.; Von Langen, D.; Yueh, W.; Tolman, R.; Heck, J.; Liebowitz, M.; Berger, J.; Doebber, T.; Zhang, B.; Moller, D.; Smith, R.; Book of Abstracts, 218th ACS National Meeting, New Orleans, **1999**, Aug., MEDI-147.

[130] De La Brouse-Elwood, F.; Jaen, J.; McGee, L.; Miao, S.; Rubenstein, S.; Chen, J.; Cushing, T.; Flygare, J.; Houze, J.; Kearney, P. WO 9938845, **1999**.

[131] Elbrecht, A.; Chen, Y.; Adams, A.; Berger, J.; Griffin, P.; Klatt, T.; Zhang, B.; Menke, J.; Zhou, G.; Smith, R.; Moller, D. *J. Biol. Chem.* **1999**, *274*, 7913.

[132] Oberfield, J.; Collins, J.; Holmes, C.; Goreham, D.; Cooper, J.; Cobb, J.; Lenhard, J.; Hull-Ryde, E.; Mohr, C.; Blanchard, S.; Parks, D.; Moore, L.; Lehmann, J.; Plunket, K.; Miller, A.; Milburn, M.; Kliewer, S.; Willson, T. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 6102.

[133] Auboeuf, D.; Rieusset, J.; Fajas, L.; Vallier, P.; Frering, V.; Riou, J.; Staels, B.; Auwerx, J.; Laville, M.; Vidal, H. *Diabetes* **1997**, *46*, 1319.

[134] (a) Alegret, M.; Cerqueda, E.; Ferrando, R.; Vazquez, M.; Sanchez, R.; Adzet, T.; Merlos, M.; Laguna, J. *Br. J. Pharmacol.* **1995**, *114*, 1351. (b) Vazquez, M.; Munoz, S.; Alegret, M.; Adzet, T.; Merlos, M.; Laguna, J. *Br. J. Pharmacol.* **1995**, *116*, 2067.

[135] Torra, I.; Gervois, P.; Staels, B. *Curr. Opin. Lipidol.* **1999**, *10*, 151.

[136] Brown, P.; Winegar, D.; Plunket, K.; Moore, L.; Lewis, M.; Wilson, J.; Sundseth, S.; Koble, C.; Wu, Z.; Chapman, J.; Lehmann, J.; Kliewer, S.; Willson, T. *J. Med. Chem.* **1999**, *42*, 3785.

[137] Marx, N.; Sukhova, G.; Collins, T.; Libby, P.; Plutzky, J. *Circulation* **1999**, *99*, 3125.

[138] Berger, J.; Leibowitz, M.; Doebber, T.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C.; Hayes, N.; Li, Y.; Marquis, R.; Santini, C.; Sahoo, S.; Tolman, R.; Smith, R.; Moller, D. *J. Biol. Chem.* **1999**, *274*, 6718.

[139] (a) Thacher, S.; Vasudevan, J.; Chandraratna, R. *Curr. Pharm. Des.* **2000**, *6*(1), 25. (b) Nagpal, S.; Chandraratna, R. *Curr. Pharm. Des.* **2000**, *6*(9), 919. (c) Chu-Moyer, M. Expert *Opin. Ther. Pat.* **2000**, *10*(6), 787. (d) Paterniti, J.; Mukherjee, R. *Pennington Cent. Nutr. Ser.* **1999**, *9*(Nutr., Genetics, and Obes.), 477.

[140] Rose, M.; Paulik, M.; Lenhard, J. *Expert Opin. Ther. Pat.* **1999**, *9*, 1223.

[141] Mukherjee, R.; Davies, P.; Crombie, D.; Bischoff, E.; Cesario, R.; Jow, L.; Hamann, L.; Boehm, M.;

Mondon, C.; Nadzan, A.; Paterniti, J.; Heyman, R. *Nature* **1997**, *386*(6623), 407.

[142] Martin, G.; Poirier, H.; Hennuyer, N.; Crombie, D.; Fruchart, J.; Heyman, R.; Besnard, P.; Auwerx, J. J. Biol. Chem. 2000, 275(17), 12612.

[143] Pfahl, M.; Lernhardt, W.; Fanjol, A. WO 9842340, **1998**.

[144] Murray, A.; Hansen, J. WO 9958486, **1999**, 37pp.

[145] Koch, S.; Dardashti, L.; Cesario, R.; Croston, G.; Boehm, M.; Heyman, R.; Nadzan, A.; *J. Med. Chem.* **1999**, *42*, 742.

[146] Momose, Y.; Odaka, H.; Imoto, H.; Kimura, H.; Sakamoto, J. WO 9958510 1999, 148pp.

[147] Sugiyama, Y.; Momose, Y.; Kimura, H.; Sakamoto, J.; Odaka, H. WO 0001679 2000, 122pp.

[148] Vuligonda, V.; Garst, M.; Chandraratna, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*(4), 589.

[149] Lenhard, J.; Lancaster, M.; Paulik, M.; Weiel, J.; Binz, J.; Sundseth, S.; Gaskill, B.; Lightfoot, R.; Brown, H. *Diabetologia* **1999**, *42*(5), 545.

[150] Rizvi, N.; Marshall, J.; Dahut, W.; Ness, E.; Truglia, J.; Loewen, G.; Gill, G.; Ulm, E.; Geiser, R.; Jaunakais, D.; Hawkins, M. *Clin. Cancer Res.* **1999**, *5*, 1658.

[151] Yoshitomi, H.; Yamazaki, K.; Abe, S.; Tanaka, I. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 85.

[152] Kumar, M.; Moore, R.; Scarpace, P. *Eu. J. Physiology* **1999**, *438*, 681.

[153] Sum, F.; Gilbert, A.; Venkatesan, A.; Lim, K.; Wong, V.; O'Dell, M.; Francisco, G.; Chen, Z.; Grosu, G.; Baker, J.; Ellingboe, J.; Malamas, M.; Gunawan, I.; Primeau, J.; Largis, E.; Steiner, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1921.

[154] Tamai, T.; Tanaka, N.; Mukaiyama, H.; Hirabayashi, A.; Muranaka, H.; Sato, M.; Akahane, M. WO 9952856, **1999**.

[155] Harada, H. .; Kato, S.; Kawashima, H.; Furutani, Y. Book of Abstracts, 214th ACS National Meeting, Las Vegas, **1997**, August, MEDI-208.

[156] Mathvink, R.; Barritta, A.; Candelore, M.; Cascieri, M.; Deng, L.; Tota, L.; Strader, C.; Wyvratt, M.; Fisher, M.; Weber, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1869.

[157] Naylor, E.; Parmee, E.; Colandrea, V.; Perkins, L.; Brockunier, L.; Candelore, M.; Cascieri, M.; Colwell Jr. L.; Deng, L.; Feeney, W.; Forrest, M.; Hom, G.; MacIntyre, D.; Strader, C.; Tota, L.; Wang, P.; Wyvratt, M.; Fisher, M.; Weber, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 755.

[158] Parmee, E.; Naylor, E.; Perkins, L.; Colandrea, V.; Ok, H.; Candelore, M.; Cascieri, M.; Deng, L.; Feeney, W.; Forrest, M.; Hom, G.; MacIntyre, D.; Miller, R.; Stearns, R.; Strader, C.; Tota, L.; Wyvratt, M.; Fisher, M.; Weber, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 749.

[159] Shih, T.; Candelore, M.; Cascieri, M.; Chiu, S.; Colwell Jr, L.; Deng, L.; Feeney, W.; Forrest, M.; Hom, G.; MacIntyre, D.; Miller, R.; Stearns, R.; Strader, C.; Tota, L.; Wyvratt, M.; Fisher, M.; Weber, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1251.

[160] Kiso, T.; Namikawa, T.; Tokunaga, T.; Sawada, K.; Kakita, T.; Shogaki, T.; Ohtsubo, Y. *Bio. Pharm. Bull.* **1999**, *22*, 1073.

[161] For a recent review of this area, see: (a) Kennedy, B. P.; Ramachandran, C. *Biochem. Pharmacol.* **2000**, *60*, 877. (b) Moller, N.; Iversen, L.; Andersen, H.; McCormack, J. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*(5), 527.

[162] Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544.

[163] Klaman, L.; Boss, O.; Peroni, O.; Kim, J.; Martino, J.; Zabolotny, J.; Moghal, N.; Lubkin, M.; Kim, Y.; Sharpe, A.; Stricker-Krongrad, A.; Shulman, G.; Neel, B.; Kahn, B. *Mol. Cell. Biol.* **2000**, *20*(15), 5479.

[164] Huyer, G.; Liu, S.; Kelly, J.; Moffat, J.; Payette, P.; Kennedy, B.; Tsaprailis, G.; Gresser, M.; Ramachandran, C. *J. Biol. Chem.* **1997**, *272*(2), 843.

[165] Cunic, C.; Detich, N.; Ethier, D.; Tracy, A. S.; Gresser, M. J.; Ramachandran, C. *J. Biol. Inorg. Chem.* **1999**, *4*, 354.

[166] Crans, D. C. *ACS Symp. Ser.* **1998**, *711*, 82.

[167] (a) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M. E.; Kahn, C. R. *Mol. Cell. Biochem.* **1995**, *53*, 217. (b) Cohen, N.; Halberstsam, M.; Shlimovich, P.; Chang, C. J.; Shamoon, H.; Rossetti, L. *J. Clin. Invest.* **1995**, *95*, 2501.

[168] Zhang, Z. Y.; MacLean, D.; McNamara, D. J.; Sawyer, T. K.; Dixon, J. E. *Biochemistry* **1994**, *33*, 2285.

[169] Huyer, G.; Kelly, J.; Moffat, J.; Zamboni, R.; Jia, Z.; Gresser, M. J.; Ramachandran, C. *Anal. Biochem.* **1998**, *258*, 19.

[170] Desmairais, S.; Jia, Z.; Ramachandran, C. *Arch. Biochem. Biophys.* **1998**, *354*, 225.

[171] Hiriyanna, K. T.; Baedke, D.; Baek, K. H.; Forney, B. A.; Kordiyak, G.; Ingribsten, T. S. *Anal. Biochem.* **1994**, *223*, 51.

[172] Burke, T. R.; Hole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Comm.* **1994**, *204*, 129.

[173] (a) Kole, H. K.; Akamatsu, M.; Ye, B.; Yan, X.; Barford, D.; Roller, P. P.; Burke, T. R. Biochem. Biophys. Res. Comm. 1995, 209, 817. (b) Burke, T. R.;Ye, B.; Akamatsu, M.; Ford, H. Jr.; Yan, X.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. P. *J. Med. Chem.* **1996**, *39*, 1021.

[174] Taing, M.; Keng, Y. F.; Shen, K.; Wu, L.; Lawrence, D. S.; Zhang, Z. Y. *Biochemistry* **1999**, *38*, 3793.

[175] Wrobel, J.; Sredy, J.; Moxham, C.; Dietrich, A.; Li, Z.; Sawicki, D. R.; Seestaller, L.; Wu, L.; Katz, A.; Sullivan, D.; Tio, C.; Zhang, Z. -Y. *J. Med. Chem.* **1999**, *42*, 3199.

[176] Harrison, S.; Ring, D. US 6057286, **2000**, 12pp.

[177] Woodgett, S. *Trends Biochem. Sci.* **1991**, *16*, 177.

[178] Bollen, M.; Keppens, S.; Stalmans, W. *Biochem. J.* **1998**, *336*, 19.

[179] (a) Embi, J. *Eur. J. Biochem.* **1980**, *107*, 519. (b) Cross, D. *Biochem. J.* **1994**, *303*, 21.

[180] Srivastava, A.; Pandey, S.; *Mol. and Cellular Biochem.* **1998**, *182*, 135.

[181] Nikoulina, S.; Ciaraldi, T.; Mudaliar, S.; Mohideen, P.; Carter, L.; Henry, R. *Diabetes* **2000**, *49*(2), 263.

[182] Eldar-Finkelman, H.; Krebs, E. *PNAS* **1997**, *94*(18), 9660.

[183] Eldar-Finkelman, H.; Schreyer, S.; Shinohara, M.; LeBoeuf, R.; Krebs, E. *Diabetes* **1999**, *48*, 1662.

[184] Summers, S.; Kao, A; Kohn, A.; Backus, G.; Roth, R.; Pessin, J.; Birnbaum, M. *J. Biol. Chem.* **1999**, *274*(25), 17934.

[185] Klien, P.; Melton, D. *PNAS* **1996**, *93*, 8455.

[186] Meijer, L.; Thunnissen, A.; White, A.; Garnier, M.; Nikolic, M.; Tsai, L.; Walter, J.; Cleverley, K.; Salinas, P.; Wu, Y.; Biernat, J.; Mandelkow, E.; Kim, S.; Pettit, G. *Chem. &Biol.* **2000**, *7*, 51.

[187] Hers, I.; Tavare, J.; Denton, R. M. *FEBS Lett.* **1999**, *460*, 433.

[188] (a) Coghlan, M.; Holder, J.; Reith, A.; Smith, D. WO 0038675, **2000**, 28 pp. (b) Coghlan, M.; Fenwick, A.; Haigh, D.; Julie, C.; Ife, R.; Reith, A.; Smith, D.; Ward, R. WO 0021927, **2000**, 131 pp.

[189] (a) Coghlan, M.; Culbert, A.; Cross, D.; Corcoran, S.; Yates, J.; Pearce, N.; Rausch, O.; Murphy, G,; Carter, P.; Cox, L.; Mills, D.; Brown, M.; Haigh, D.; Ward, R.; Smith, D.; Murray, K.; Reith, A.; Holder, J. *Chem. &Biol.* **2000**, *24*, 1. (b) Coghlan, M.; Holder, J.; Reith, A.; Smith, D. WO 0038675, **2000**, 28 pp.

[190] (a) Watanabe, K.; Ando, R.; Saito, K.; Kawamoto, R.; Shoda, A. WO 0018758, **2000**, 106 pp. (b) Shoda, A.; Watanabe, K.; Aritomo, K.; Jikihara, T.; Ando, R.; Saito, K.; Honda, T.; Kawamoto, R. WO 0017184, **2000**, 151 pp.

[191] Nuss, J.; Harrison, S.; Ring, D.; Boyce, R.; Brown, S.; Goff, D.; Johnson, K.; Pfister, K.; Ramurthy, S.; Renhowe, P.; Seely, L.; Subramanian, S.; Wagman, A.; Zhou, X. WO 9965897, **1999**.

[192] Shaw, M.; Cohen, P.; Alessi, D. *FEBS Lett.* **1997**, 416(3), 307.

[193] (a) Malse, R.; Rochford, J.; McCormack, J.; Vandenheede, J.; Hemmings, B.; Yeaman, S. *J. Biol. Chem.* **1999**, *274*(2), 776. (b) Brady, M.; Bourbonais, F.; Saltiel, A. *J. Biol. Chem.* **1998**, *273*(23), 14063.

[194] Cohen, P.; Alessi, D.; Cross, D. WO 9722360, 1997, 98 pp.

[195] Arce, A.; Aicher, L.; Wahl, D.; Anderson, N.; Meheus, L.; Raymackers, J.; Cordier, A.; Steiner, S. *Life Sci.* **1998**, *63*, 2243.

[196] Duhault, J.; Berger, S.; Boulanger, M.; Della, Z.; Lacour, F.; Wierzbicki, M. *Arzneimittelforschung* **1998**, *48*, 734.

[197] Nagao, Y.; Konno, F.; Kotake, J.; Ishii, F.; Honda, H.; Sato, S. EP 903343, **1999**.

[198] Bedoya-Zurita, M.; Diaz-Martin, J.; Daumas, M. WO 9952876, **1999**.

[199] Aasum, E. *Circulation* **1999**, *100*(18, Suppl. I): Abst. 633.

[200] Yamasaki, N.; Imoto, T.; Oku, T.; Kayakiri, H.; Onomura, O.; Hiramura, T. WO 9951574, **1999**, 88pp.

[201] (a) Yamasaki, N.; Imoto, T.; Hiramura, T.; Kawauchi, M.; Oku, T.; Kayakiri, H.; Sawada, H.; Abe, Y.; Hamashima, H.; Ishibashi, N.; Setoi, H. WO 0039099 **2000**, 154pp. (b) Kayakiri, H.; Abe, Y.; Hamashima, H.; Sawada, H.; Ishibashi, N.; Setoi, H.; Oku, T.; Yamasaki, N.; Imoto, T.; Hiramura, T. WO 0039097 **2000**, 140 pp. (c) Kayakiri, H.; Abe, Y.; Hamashima, H.; Sawada, H.; Ishibashi, N.; Setoi, H.; Oku, T.; Yamasaki, N.; Imoto, T.; Hiramura, T. WO 0034277 **2000**, 226 pp.

[202] Goke, B.; Herrmann-Rinke, C. *Diabetes/Metab. Rev.* **1998**, *14*(Suppl. 1), S31-S38.

[203] Scheen, A. *Diabetes Metab.* **1998**, *24*, 311.

[204] Hanefeld, M. *J. Diabetes Complications* **1998**, *12*, 228.

[205] Scorpiglione, N.; Belfiglio, M.; Carinci, F.; Cavaliere, D.; DeCurtis, A.; Franciosi, M.; Mari, E.; Sacco, M.; Tognoni, G.; Nicolucci, A. *Eur. J. Clin. Pharmacol.* **1999**, *55*, 239.

[206] Holman, R.; Cull, C.; Turner, R. *Diabetes Care* **1999**, *22*, 960.

[207] Wolever, T.; Chiasson, J.; Josse, R.; Hunt, J.; Palmason, C.; Rodger, N.; Ross, S.; Ryan, E.; Tam, M. *Diabetes Care* **1998**, *21*, 1612.

[208] Matsumoto, K.; Yano, M.; Miyake, S.; Ueki, Y.; Yamaguchi, Y.; Akazawa, S.; Tominaga, Y.; *Diabetes Care* **1998**, *21*, 256.

[209] Ishida, H.; Kato, S.; Nishimura, M.; Mizuno, N.; Fujimoto, S.; Mukai, E.; Kajikawa, M.; Yamada, Y.; Odaka, H.; Ikeda, H.; Seino, Y. *Horm. Metab. Res.* **1998**, *30*, 673.

[210] Sels, J.; Huijberts, M.; Wolffenbuttel, B. *Expert Opin. Pharmacother.* **1999**, *1*, 149.

[211] Asano, N.; Nash, R.; Molyneux, R.; Fleet, George W. *Tetrahedron Asym.* **2000**, *11*(8), 1645.

[212] Schmitz, O. *Diabetes Annu.* **1999**, *12*, 269.

[213] Scherbaum, W. *Exp. Clin. Endocrinol. Diabetes* **1998**, *106*, 97.

[214] Amylin, *Product Pipeline* **2000**.

[215] Ebenezer, I. *Meth. Find. Exp. Clin. Pharmacol.* **1999**, *21*, 167.

[216] Lee, W.; Kanai, Y.; Wells, R.; Hediger, M.; *J. Biol. Chem.* **1994**, *269*, 12032.

[217] Wells, R.; Pajor, A.; Kanai, Y.;Turk, E.; Wright, E.; Hediger, M. *Am. J. Physiol.* **1992**, *263*, F459.

[218] Hediger, M.; Kanai, Y.; You, G.; Nussberger, S. *J. Physiol* **1995**, *482*, 7S.

[219] You, G.; Lee, W.; Barros, E.; Kanai, Y.; Huo, T.; Khawaja, S.; Wells, R.; Nigam, S.; Hediger, M. *J. Biol. Chem.* **1995**, *270*, 29365.

[220] Kong, C.; Yet, S.; Lever, J. *J. Biol. Chem.* **1993**, *268*, 1509.

[221] Kanai, Y.; Lee, W.; You, G.; Brown, D.; Hediger, M. *J. Clin. Invest.* **1994**, *93*(1), 397.

[222] Lotspeich, W. *Harvey Lect.* **1961**, *56*, 63.

[223] (a) Shi, Z.; Rastogi, K.; Lekas, M.; Efendic, S.; Drucker, D.; Vranic, M. *Endocrinology* **1996**, *137*(8), 3193-3199. (b) Starke, A.; Grundy, S.; McGarry, J.; Unger, R. *Proc. Natl. Acad. Sci. USA* **1985**, *82*(5), 1544.

[224] Lin, J.; Hahn, K.; Kline, R. *Biochim. Biophys. Acta* **1982**, *693*(2), 379.

[225] Dyer, J.; Nash, R.; Shirazi-Beechey, S. *Biochem. Soc. Trans.* **1998**, *26*(2), S180.

[226] Tsujihara, K.; Hongu, M.; Saito, K.; Inamasu, M.; Arakawa, K.; Oku, A.; Matsumoto, M. *Chem. Pharm. Bull.* **1996**, *44*(6), 1174.

[227] Tsujihara, K.; Hongu, M.; Saito, K.; Kawanishi, H.; Kuriyama, K.; Matsumoto, M.; Oku, A.; Ueta, K.; Tsuda, M.; Saito, A. *J. Med. Chem.* **1999**, *42*, 5311.

[228] Oku, A.; Ueta, K.; Arakawa, K.; Ishihara, T.; Nawano, M.; Kuronuma, Y.; Matsumoto, M.; Saito, K.; Tsujihara, K.; Anai, M.; Asano, T.; Kanai, Y. Endou, H. *Diabetes* **1999**, *48*, 1794.

[229] Oku, A.; Ueta, K.; Nawano, M.; Arakawa, K.; Kano-Ishihara, T.; Matsumoto, M.; Saito, A.; Tsujihara, K.; Anai, M.; Asano, T. *Eur. J. Pharm.* **2000**, *391*, 183.

[230] Thompson, K.; McNeill, J.; Orvig, C. *Chem. Rev.* **1999**, *99*, 2561.

[231] Cincotta, A.; Meier, A.; Cincotta, M. *Expert Opin. Invest. Drugs* **1999**, *8*, 1683.

[232] Scislowski, P.; Tozzo, E.; Zhang, Y.; Phaneuf, S.; Prevelige, R.; Cincotta, A. *Int. J. Obes.* **1999**, *23*, 425.

[233] Costantino, L.; Rastelli, G.; Vianello, P.; Cignarella, G.; Barlocco, D. *Med. Res. Rev.* **1999**, *19*, 3.

[234] Oates, P.; Mylari, B.; *Expert Opin. Invest. Drugs* **1999**, *8*, 2095.

[235] Schmidt, R.; Dorsey, D.; Beaudet, L.; Plurad, S.; Williamson, J.; Ido, Y. *J. Neuropathol. Exp. Neurol.* **1998**, *57*, 1175.

[236] Kador, P.; Inoue, J.; Secchi, E.; Lizak, M.; Rodriguez, L.; Mori, K.; Greentree, W.; Blessing, K.; Lackner, P.; Sato, S. *Exp. Eye Res.* **1998**, *67*, 203.

[237] Oates, P.; Ellery, C.; *Diabetologia* **1999**, *42*(Suppl. 1): Abst 1043.

[238] Niwa, T.J.; Chromatogr. B. Biomed. Sci. Appl. **1999**, 731, 23.

[239] Lamour, V.; Barth, P.; Rogniaux, H.; Poterszman, A.; Howard, E.; Mitschler, A.; Van Dorsselaer, A.; Podjarny, A.; Moras, D. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1999**, *D55*, 721.

[240] Banditelli, S.; Boldrini, E.; Vilardo, P.; Cecconi, I.; Cappiello, M.; Dal Monte, M.; Marini, I.; Del Corso, A.; Mura, U. *Exp. Eye Res.* **1999**, *69*, 533.

[241] Jacot, J.; Hosotani, H.; Glover, J.; Lois, N.; Robison, W. *J. Ocul. Pharmacol. Ther.* **1998**, *14*, 169.

[242] Neuenschwander, H.; Takahashi, Y.; Kador, P. *J. Ocul. Pharmacol. Ther.* **1997**, *13*, 517.

[243] Hotta, N.; Koh, N.; Sakakibara, F.; Nakamura, J.; Hara, T.; Hamada, Y.; Fukasawa, H.; Kakuta, H.; Sakamoto, N. *Eur. J. Pharmacol.* **1997**, *326*, 45.

[244] Ashizawa, N.; Yoshida, M.; Sugiyama, Y.; Akaike, N.; Ohbayashi, S.; Aotsuka, T.; Abe, N.; Fukushima, K.; Matsuura, A. *Jpn. J. Pharmacol.* **1997**, *73*, 133.

[245] Kubo, E.; Nakamura, S.; Tsuzuki, S.; Takahashi, Y.; Akagi, Y.; Mizuno, K.; Matsubara, A.; *Graefe's Arch. Clin. Exp. Ophthalmol.* **1999**, *237*, 758.

[246] Ueno, R.; Kato, I. WO 9938497, **1999**.

[247] Carrington, A. *Diabetologia* **1999**, *42*(Suppl. 1): Abst 1108.

[248] Efanova, I. *Curr. Opin. Oncol., Endocr. Metab. Invest. Drugs* **1999**, 1, 101.

[249] Negoro, T.; Murata, M.; Ueda, S.; Fujitani, B.; Ono, Y; Kuromiya, A.; Komiya, M.; K.; Suzuki, Matsumoto, J. *J. Med. Chem.* **1998**, *41*, 4118.

[250] Jones, M.; Gunn, D.; Jones, J.; Van Zandt, M. WO 9950268, **1999**.

[251] Reymundo Cuesta, I.; Martin Lopez, M.; Garcia De Quesada Fort, T.; De La Fuente Blanco, J. *PCT Int. Appl.* **2000**, 22 pp.

[252] Oka, M.; Matsumoto, Y.; Sugiyama, S.; Tsuruta, N.; Matsushima, M. *J. Med. Chem.* **2000**, *43*(12), 2479.

[253] Costantino, L.; Rastelli, G.; Gamberini, M.; Barlocco, D. *Expert Opin. Ther. Pat.* **2000**, *10*(8), 1245.

[254] Hong, S.; Lee, K.; Handa, J.; Joo, C. *Biochem. Biophys. Res. Commun.* **2000**, *275*(1), 53.

[255] (a) Raj, D.; Choudhury, D.; Welbourne, T.; Levi, M. *Am. J. Kidney Dis.* **2000**, *35*(3), 365. (b) Brownlee, M. *Metab., Clin. Exp.* **2000**, *49*(2, Suppl. 1), 9.

[256] Ruggiero-Lopez, D.; Lecomte, M.; Moinet, G.; Patereau, G.; Lagarde, M.; Wiernsperger, N. *Biochem. Pharmacol.* **1999**, *58*, 1765.

[257] Simpson, A.; Jones, R. *Life Sci.* **1999**, *64*, 1427.

[258] Thornalley, P.; Yurek-George, A.; Argirov, O. *Biochem. Pharmacol.* **2000**, *60*(1), 55.

[259] Piercy, V.; Toseland, C.; Turner, N. *Metab., Clin. Exp.* **1998**, *47*, 1477.

[260] Birrell, A.; Heffernan, S.; Ansselin, A.; McLennan, S.; Church, D.; Gillin, A.; Yue, D. *Diabetologia* **2000**, *43*(1), 110.

[261] Tanaka, Y.; Inoue, K.; Shimada, S.; Shimizu, T.; Kawasumi, M.; Iwamoto, H.; Onuma, T.; Kawamori, R. *Curr. Ther. Res.* **2000**, *61*(4), 191.

[262] Oturai, P.; Christensen, M.; Rolin, B.; Pedersen, K.; Mortensen, S.; Boel, E. *Metab., Clin. Exp.* **2000**, *49*(8), 996.

[263] Cooper, M. E.; Thallas, V.; Forbes, J.; Scalbert, E.; Sastra, S.; Darby, I.; Soulis, T. *Diabetologia* **2000**, *43*(5), 660.

[264] Rellier, N.; Ruggiero-Lopez, D.; Lecomte, M.; Lagarde, M.; Wiernsperger, N. *Life Sci.* **1999**, *64*, 1571.

[265] Burnier, M.; Brunner, H. *J. Am. Soc. Nephrol.* **1999**, *10*(4, Suppl. 12), S278.

[266] Mogyorosi, A.; Sonkodi, S. *Diabetes/ Metab. Res. Rev.* **1999**, *15*, 55.

[267] Singh, R.; Alavi, N.; Singh, A.; Leehey, D. *Diabetes* **1999**, *48*, 2066.

[268] Kedziora-Kornatowska, K. *Clin. Chim. Acta* **1999**, *287*, 19.

[269] Kunt, T.; Forst, T.; Pfutzner, A.; Beyer, J.; Wahren, J. *Pathophysiology* **1999**, *5*, 257.

[270] Nakamura, J.; Kato, K.; Hamada, Y.; Nakayama, M.; Chaya, S.; Nakashima, E.; Naruse, K.; Kasuya, Y.; Mizubayashi, R.; Miwa, K.; Yasuda, Y.; Kamiya, H.; Ienaga, K.; Sakakibara, F.; Koh, N.; Hotta, N. *Diabetes* **1999**, *48*, 2090.

[271] Kapor-Drezgic, J.; Zhou, X.; Babazono, T.; Dlugosz, J.; Hohman, T.; Whiteside, C. *J. Am. Soc. Nephrol.* **1999**, *10*, 1193.

[272] Dagogo-Jack, S.; Santiago, J. *Arch. Intern. Med.* **1997**, *157*, 1802.

[273] Dallongeville, J.; Fruchart, J.; Auwerx, J. *J. Med. Chem.* **1998**, *41*, 5337.

[274] Uusitupa, M. *Curr. Opin. Lipidol.* **1999**, *10*, 3.

[275] (a) Welsh, N. *Cur. Opin. Mol. Therap.* **1999**, *1*, 464. (b) Freeman, D.; Leclerc, I.; Rutter, G. *Int. J. Mol. Med.* **1999**, *4*, 585.

EXHIBIT C

Oral Antihyperglycemic Therapy for Type 2 Diabetes

Scientific Review

Silvio E. Inzucchi, MD

Context Care of patients with type 2 diabetes has been revolutionized throughout the past several years—first, by the realization of the importance of tight glycemic control in forestalling complications, and second, by the availability of several unique classes of oral antidiabetic agents. Deciphering which agent to use in certain clinical situations is a new dilemma facing the primary care physician.

Objective To systematically review available data from the literature regarding the efficacy of oral antidiabetic agents, both as monotherapy and in combination.

Data Sources A MEDLINE search was performed to identify all English-language reports of unique, randomized controlled clinical trials involving recently available oral agents for type 2 diabetes. Bibliographies were also reviewed to find additional reports not otherwise identified.

Study Selection and Data Extraction Studies (63) were included in the analysis if they had a study period of at least 3 months; if each group contained at least 10 subjects at the study's conclusion; and if hemoglobin A_{1c} was reported. When multiple dosages of a drug were tested, the results of the highest approved dosage were used. In placebo-controlled trials, hemoglobin A_{1c} data are presented as the difference between the change in treated vs placebo subjects.

Data Synthesis Five distinct oral drug classes are now available for the treatment of type 2 diabetes. Compared with placebo treatment, most of these agents lower hemoglobin A_{1c} levels approximately 1% to 2%. Equivalent efficacy is usually demonstrated when different agents are compared with one another in the same study population. When they are used in combination, there are additional glycemic benefits. Long-term vascular risk reduction has been demonstrated only with sulfonylureas and metformin.

Conclusions With few exceptions, the available oral antidiabetic agents are equally effective at lowering glucose concentrations. Their mechanisms of action are different, however, and as a result they appear to have distinct metabolic effects. These are reflected in their adverse effect profiles and their effect on cardiovascular risk, which may influence drug choice.

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EPIDEMIOLOGY

Diabetes mellitus affects more than 6% of the US population, with the great majority having type 2 diabetes mellitus (DM).[1] In some older groups, the prevalence of DM and its metabolic forerunner, impaired glucose tolerance (IGT), approaches 25%.[2] Throughout the past decade, a 30% increase in the prevalence of DM has been recorded in the United States, with the most dramatic increases in younger individuals.[3] When the long-term complications of this disease and their costs are considered, the implications of these statistics are sobering.[4]

The importance of blood glucose control in preventing microvascular complications of DM, such as retinopathy and nephropathy, is now recognized.[5-7] Whether such a relationship exists for macrovascular complications, such as myocardial infarction and stroke, is less clear.[7] Simultaneously, a rapidly expanding therapeutic armamentarium is now available to treat hyperglycemia in type 2 DM. The number of oral antihyperglycemic agent classes, each with its unique mechanism of action, has increased 5-fold throughout the past 6 years—an often confusing increase in new categories of drugs: biguanides, α-glucosidase inhibitors, thiazolidinediones (TZDs), and nonsulfonylurea insulin secretagogues.

See also pp 373 and 379.

More therapeutic options translate into more complex decision making for primary care physicians and diabetic patients. In this article I review the individual oral-agent drug classes and the published evidence demonstrating their

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efficacy in lowering glucose concentrations as well as their effectiveness in preventing diabetic complications.

METHODS

A MEDLINE search was performed to identify all English-language articles of unique, randomized controlled clinical trials involving recently available oral agents for type 2 DM. Bibliographies were also reviewed to find additional reports not otherwise identified. Studies (63) were included in the analysis if they met the following criteria: study period of at least 3 months, each group containing at least 10 subjects at the study's conclusion, and hemoglobin A_{1c} (HbA_{1c}) reported. When multiple doses of a drug were tested, the results of the highest approved dose were used. In placebo-controlled trials, HbA_{1c} data are presented by convention as the difference between the change in treated vs placebo subjects.

PATHOGENESIS OF TYPE 2 DM

Knowledge of the pathogenesis of type 2 DM is important in understanding the appropriate role for each oral-agent class. Type 2 DM is a complex metabolic disorder resulting from relatively decreased pancreatic insulin secretion and variable contributions of decreased insulin action, or insulin resistance, in target tissues, mainly muscle and the liver.[8,9] Insulin resistance is first demonstrated in skeletal muscle, in which higher concentrations of insulin are necessary to allow glucose to enter cells. Peripheral insulin resistance predicts the development of type 2 DM[9,10] and is detected in normoglycemic first-degree relatives of patients with type 2 DM.[11-13] It is influenced by both genetic and environmental (eg, obesity) factors. Insulin-resistant individuals frequently exhibit a constellation of other characteristics, including visceral obesity, dyslipidemia, hypertension, hyperinsulinemia, impaired fibrinolysis, endothelial dysfunction, hyperuricemia, vascular inflammation, and premature atherosclerosis.[14] They are said to have the metabolic syndrome,[15] or insulin resistance syndrome, emphasizing the presumed central pathogenic role of insulin resistance.

Initially, in the face of insulin resistance, compensatory increases in pancreatic insulin secretion are able to maintain normal glucose concentrations. However, as the disease progresses, insulin production gradually diminishes, leading to progressive stages of hyperglycemia. Hyperglycemia is first exhibited in the postprandial state, since uptake by skeletal muscle is the metabolic fate of the majority of ingested carbohydrate energy, and then during fasting. As insulin secretion decreases, hepatic glucose production, normally attenuated by insulin, increases. This increase is primarily responsible for the elevation of fasting glucose levels in patients with type 2 DM. Superimposed upon these mechanisms is the well-recognized deleterious effect of hyperglycemia itself—glucotoxicity—upon both insulin sensitivity and insulin secretion.[16]

Adipose tissue plays an important but often overlooked role in the pathogenesis of type 2 DM. Insulin resistance is also demonstrated at the adipocyte level, leading to unrestrained lipolysis and elevation of circulating free fatty acids. Increased free fatty acids, in turn, further dampens the insulin response in skeletal muscle[17,18] while further impairing pancreatic insulin secretion as well as augmenting hepatic glucose production ("lipotoxicity").[19]

Therefore, type 2 DM results from coexisting defects at multiple organ sites: resistance to insulin action in muscle, defective pancreatic insulin secretion, and unrestrained hepatic glucose production, all of which are worsened by defective insulin action in fat (FIGURE). These pathophysiological lesions are to blame for the development and progression of hyperglycemia. They are also the primary targets for pharmacological therapy.

THE IMPORTANCE OF GLYCEMIC CONTROL

The American Diabetes Association's recommended targets for glycemic control include a preprandial blood glucose level of 80 to 120 mg/dL (4.4 to 6.7 mmol/L), a bedtime blood glucose level of 100 to 140 mg/dL (5.6 to 7.8 mmol/L), and an HbA_{1c} level of less than 7%.[20] More stringent guidelines[21] have recently been offered by the American College of Endocrinology and the American Association of Clinical Endocrinologists: preprandial blood glucose levels less than 110 mg/dL (6.1 mmol/L), 2-hour postprandial glucose levels less than 140 mg/dL (7.8 mmol/L), and HbA_{1c} levels at 6.5%. These recommendations are based on findings from 3 landmark studies: the Diabetes Control and Complications Trial,[5] the Kumamoto Study,[6] and the United Kingdom Prospective Diabetes Study (UKPDS),[7] which have shown unequivocally that maintaining blood glucose concentrations as close to normal as possible in both type 1 and type 2 DM decreases the incidence of microvascular complications.

Nonpharmacological Therapy

Diet, exercise, and weight loss are at the center of any therapeutic program. Not only do these lifestyle modifications lower blood glucose concentrations, but also they ameliorate many of the frequently coexisting risk factors for cardiovascular disease. Unfortunately, most patients are unable to achieve adequate control with lifestyle interventions alone, which should not detract from their critical role, since they enhance the effectiveness of medical regimens. A controlled-energy diet and regular aerobic exercise are therefore recommended for the majority of patients with type 2 DM, who are usually overweight.[22,23]

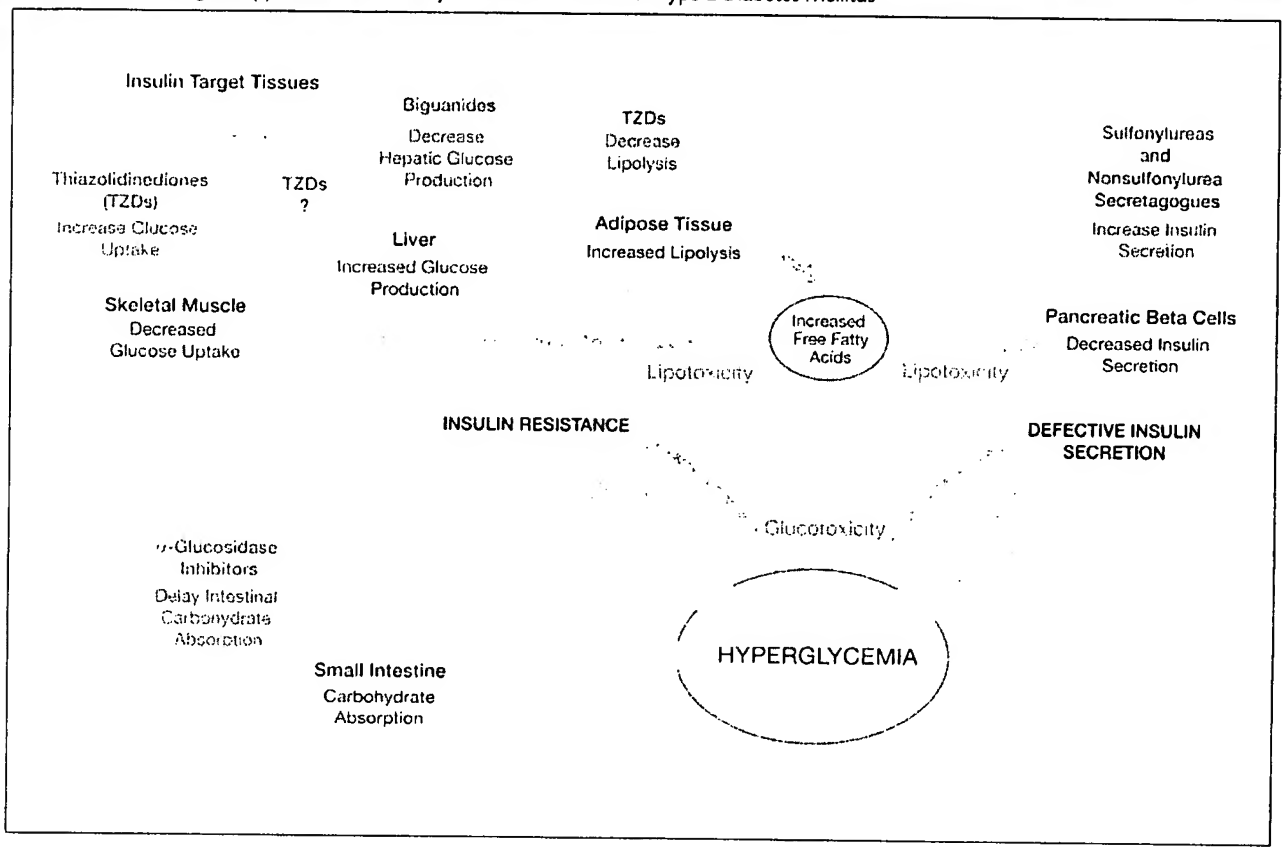
Pharmacological Therapy

Sulfonylureas. Sulfonylurea (SU) drugs have been available in the United States since 1954. Second-generation SUs (glyburide, glipizide, and glimepiride) are more potent and probably safer than first-generation SUs (chlorpropamide, tolbutamide, acetohexamide, and tolazamide) but essentially of equal efficacy.[24] The SUs bind to the SU re-

ceptor, found on the surface of pancreatic beta cells. This interaction leads to a closure of voltage-dependent potassium adenosinetriphosphate (K_{ATP}) channels, facilitating cell membrane depolarization, calcium entry into the cell, and insulin secretion.[25] Thus, SUs allow for insulin release at lower glucose thresholds than normal. They partially reverse the attenuated insulin secretion that characterizes type 2 DM. Understandably, in the face of SU therapy, circulating insulin concentrations are increased.[26] As a result, and despite the presence of insulin resistance, glucose concentrations fall. The possibility that such agents may also directly enhance peripheral glucose disposal (ie, decrease insulin resistance) has also been raised.[27,28] However, the peripheral effects of SUs are most likely secondary to a reduction in glucotoxicity.

When compared with placebo, SU therapy leads to a mean decrease in HbA_{1c} of approximately 1% to 2% (TABLE 1).[7,29,30] One study[31] demonstrated a more impressive change, but the rise in HbA_{1c} level experienced by the placebo group was greater than usual, reaching 2%. Current agents are equally efficacious[32-37] and vary subtly, such as in their metabolism and duration of action. The newest member of this class, glimepiride, binds less avidly in cardiac tissues, which contain K_{ATP} channels similar to those of beta cells. Glimepiride, therefore, may reduce ischemic preconditioning less than the other SUs do,[38] the clinical importance of which is unclear. In general, there is no consistent additional benefit on coexisting conditions, such as elevated lipid levels or blood pressure. Given the epidemiological association between hyperinsulinemia and cardiovascular disease, some have raised concerns that SUs increase cardiovascular morbidity.[39,40] An early trial by the University Group Diabetes Project,[41] which explored the effectiveness of oral agents vs insulin, found increased cardiovascular mortality in the cohort of patients randomized to SUs. Widespread criticism of the project's methodology has placed the validity of its findings in doubt.[42] In the more recent UKPDS, which had a better experimental design, increased mortality was not shown in SU-treated subjects.[7] Given these agents' mechanism of action and frequent loss of efficacy over time, another concern is their potential to exhaust beta cell function. However, as demonstrated in the UKPDS, the inexorable decline in beta cell function may be an underlying characteristic of the diabetic state itself, independent of treatment modality. Of more practical concern, SU therapy is associated with 2 common adverse effects. The first is weight gain, typically from 2 to 5 kg, problematic in

Figure. Pharmacological Approaches to the Major Metabolic Defects of Type 2 Diabetes Mellitus



a group of patients frequently already overweight.[7,25,28,29] The second is hypoglycemia, most likely to affect the elderly, those with worsening renal function, and those with irregular meal schedules.[7,25,32]

In the UKPDS, 4209 patients newly diagnosed with type 2 DM were randomized to either intensive (medication) or conventional (diet) treatment and observed for approximately 10 years. The intensive-treatment group underwent a subsequent randomization to primary therapy with SU or insulin. When compared with conventional therapy, intensive treatment was associated with a decreased risk of predominantly microvascular complications, including a 12% reduction in any diabetes-related end point ($P=.03$) and a 25% reduction in all microvascular end points ($P<.001$). There was no significant effect on diabetes-related death or on all-cause mortality, however, and there was only a trend toward a small effect (−16%) on the risk of myocardial infarction ($P=.05$).[7] Overall, there were no significant differences between SU-treated subjects and those treated with insulin. One might argue that improved glycemic control from SUs did not significantly decrease macrovascular risk because this effect was negated by the opposing effect of hyperinsulinemia.

Optimal dosing of each member of this class varies. As a general rule, however, glucose-lowering effect plateaus after half the maximal recommended

Table 1. Antidiabetic Oral Agent Monotherapy: Randomized Controlled Clinical Trials*

Source, y	Treatment Arms	Subjects, No.	Study Length	Hemoglobin A_{1c} Reduction, %†
	Sulfonylureas			
UKPDS,[7] 1998	Sulfonylureas vs diet	3867	10 y	0.9
Schade et al,[29] 1998	Glimepiride vs placebo	249	22 wk	1.4
Simonson et al,[28] 1984	Glipizide GITS vs placebo	204	12 wk	1.8
Rosenstock et al,[31] 1996	Glimepiride vs placebo	416	14 wk	2.5
	Metformin			
UKPDS,[58] 1998	Meformin vs diet	753	10.7 y	0.8
Hoffmann and Spengler,[57] 1997	Metformin vs placebo	96	24 wk	1.1
Garber et al,[56] 1997	Metformin vs placebo	452	11 wk	2.0
Grant,[55] 1996	Metformin vs placebo	75	6 mo	1.7
DeFronzo and Goodman,[52] 1995	Metformin vs placebo	289	29 wk	1.5
Nagi and Yudkin,[54] 1993	Metformin vs placebo	27	12 wk	1.3
Dornan et al,[53] 1991	Metformin vs placebo	60	8 mo	3.0
	α-Glucosidase Inhibitors			
Hasche et al,[80] 1999	Acarbose vs placebo	74	24 mo	0.9
Scott et al,[79] 1999	Acarbose vs placebo	105	16 wk	0.4
Fischer et al,[77] 1998	Acarbose vs placebo	495	24 wk	1.0
Johnston et al,[78] 1998	Miglitol vs placebo	345	12 mo	0.7
Hoffmann and Spengler,[82] 1997	Acarbose vs placebo	96	24 wk	1.3
Braun et al,[76] 1996	Acarbose vs placebo	86	24 wk	0.9
Coniff et al,[75] 1995	Acarbose vs placebo	290	16 wk	0.8
Coniff et al,[81] 1995	Acarbose vs placebo	212	24 wk	0.6
Chiasson et al,[74] 1994	Acarbose vs placebo	354	1 y	0.9
Hotta et al,[71] 1993	Acarbose vs placebo	40	24 wk	1.0
Santeusanio et al,[72] 1993	Acarbose vs placebo	62	16 wk	0.6
Hanefeld et al,[70] 1991	Acarbose vs placebo	94	24 wk	0.6
	Thiazolidinediones			
Lebovitz et al,[100] 2001	Rosiglitazone vs placebo	493	26 wk	1.5
Phillips et al,[99] 2001	Rosiglitazone vs placebo	959	26 wk	1.5
Aronoff et al,[101] 2000	Pioglitazone vs placebo	408	26 wk	1.6
Fonseca et al,[98] 1998	Troglitazone vs placebo	402	6 mo	1.1
	Non-SU Secretagogues			
Jovanovic et al,[126] 2000	Repaglinide vs placebo	93	6 mo	1.9
Horton et al,[134] 2000	Nateglinide vs placebo	701	24 wk	1.0
Hanefeld et al,[128] 2000	Nateglinide vs placebo	289	12 wk	0.6
Goldberg et al,[127] 1998	Repaglinide vs placebo	99	18 wk	1.7

*UKPDS indicates United Kingdom Prospective Diabetes Study; GITS, gastrointestinal transport system; and SU, sulfonylurea.
†Values represent the placebo-adjusted absolute percentage reduction in the active therapy group, adjusted for placebo. Because of different recruitment criteria for individual studies, particularly regarding baseline hemoglobin A_{1c}, direct comparison of one agent with another is difficult.

dose is reached.[30,43] Most agents undergo metabolism by the liver and are cleared by the kidney. Therefore, they must be used cautiously in those with advanced forms of either hepatic or renal impairment. Sulfonylureas are approved for use as monotherapy and in combination with all other oral agent classes (except the non-SU secretagogues) and insulin.

Biguanides. Although available internationally for decades, metformin, a biguanide, was not released in the United States until 1995.[44] An earlier biguanide, phenformin, was removed from the market in the 1970s because of an association with lactic acidosis.[45] In contrast to the SUs, metformin does not stimulate insulin secretion.[46,47] The precise mode of action of metformin remains somewhat controversial, but its predominant effect is to reduce hepatic glucose production in the presence of insulin.[48,49] It is therefore considered an insulin sensitizer. Increased peripheral glucose disposal has also been measured,[44,50] although this is most likely a secondary phenomenon caused by lowering of glucotoxicity and not a direct effect of the drug itself.[48,51]

In placebo-controlled trials, metformin's ability to lower HbA_{1c} is similar to that of SUs (ie, −1% to 2%, placebo-adjusted) (Table 1).[52-59] When compared with SUs in head-to-head trials, metformin's glucose-lowering effect is generally equivalent (TABLE 2).[60-63] Metformin monotherapy, however, is associated with weight loss (or no weight gain) and much less hypoglycemia than SU therapy.[44,47,48] Because of the lack of beta cell stimulation, circulating insulin concentrations tend to decline, which may provide a cardiovascular advantage. Other nonglycemic benefits have also been ascribed to metformin, such as decreases in lipid levels (low-density lipoprotein cholesterol and triglycerides)[52,64] and the antifibrinolytic factor plasminogen activator inhibitor 1.[64] Recently, an amelioration in vascular reactivity or endothelial function has also been demonstrated.[65] The only study that has examined the overall effectiveness of metformin on long-term complications is the UKPDS, where the agent was included in the randomization schema with conventional diet therapy and intensive SU-insulin treatment in a subgroup of overweight patients. Those who received metformin experienced less hypoglycemia and weight gain than those who received SUs or insulin. With a similar HbA_{1c} reduction observed in the other intensively treated subjects, more impressive risk reduction was noted in the primary aggregate end points. Metformin-treated subjects, for instance, had a 32% reduction in any diabetes-related end point ($P=.02$), 42% less diabetes-related deaths ($P=.02$), and a 36% reduction in all-cause mortality ($P=.01$). Specifically, compared with that of the conventional group, the risk of myocardial infarction was reduced by 39% ($P=.01$); of all macrovascular end points, by 30% ($P=.02$).[58] Individual and total microvascular end points were not significantly reduced, however, presumably because of the relatively small sample size, since there were no differences in microvascular outcomes between the metformin and the SU–insulin-treated groups. These important findings suggested that the manner in which glucose levels are lowered by antidiabetic agents might uniquely influence certain outcomes. In addition, metformin has been shown to improve ovulatory function in insulin-resistant women with polycystic ovarian syndrome and, most recently, to decrease the progression from IGT to type 2 DM.

Adverse effects of metformin therapy include gastrointestinal distress, such as abdominal pain, nausea, and diarrhea, in up to 50% of patients.[44] The frequency of these adverse effects can be minimized with food consumption and slow titration of dose; the need to discontinue therapy is uncommon. The optimal dosage in most patients appears to be 2000 mg/d.[56] The risk of lactic acidosis is approximately 100 times less than that with phenformin therapy: approximately 1 in every 30 000 patient-years.[66] The drug must be avoided in those who are at increased risk for lactic acidosis, such as those with renal impairment (serum creatinine level ≥1.5 mg/dL [132.6 μmol/L] for men or ≥1.4 mg/dL [123.8 μmol/L] for women), in whom metformin clearance is diminished. Other contraindications include hepatic dysfunction, congestive heart failure, metabolic acidosis, dehydration, and alcoholism. It should be temporarily withheld in patients with virtually any acute illness and those undergoing surgery or radiocontrast studies. The need for additional therapies after several years of use was also demonstrated in metformin-treated subjects in the UKPDS, so beta cell failure also occurs in patients who are treated with this agent.[67] It is approved for use as monotherapy and in combination with SUs and other secretagogues, TZDs, and insulin.

α-Glucosidase Inhibitors. The α-glucosidase inhibitors (AGIs; eg, acarbose and miglitol) were introduced in 1996. Their mechanism of action is unique, and this is the sole drug class not targeted at a specific pathophysiological defect of type 2 DM. An enzyme in the brush border of the proximal small intestinal epithelium, α-glucosidase serves to break down disaccharides and more complex carbohydrates. By the competitive inhibition of this enzyme, the AGIs delay intestinal carbohydrate absorption and mitigate postprandial glucose excursions.[68,69]

The efficacy of AGIs is considerably less than that of either SUs or metformin, with an average HbA_{1c} lowering effect of approximately 0.5% to 1% compared with that of placebo-treated subjects (Table 1).[57,70-80] Not surprisingly, their greatest effect is on postprandial glucose levels, whereas the effect on fasting blood glucose levels is small.[57,70-80] In a comparative study, acarbose had about half the glucose-lowering effect of an SU, tolbutamide.[81] Several other head-to-head trials have claimed efficacy equal to that of SUs[82,83] and metformin,[57] but in 2 of these,[57,82] the dose of the comparator drug was suboptimal (Table 2).

The AGIs are attractive in that they are essentially nonsystemic and unas-

sociated with hypoglycemia and weight gain. Nonglycemic benefits include small reductions in triglycerides and postprandial insulin levels.[69,84] The targeting of postprandial glucose may provide a theoretical advantage because postprandial hyperglycemia has been linked with cardiovascular mortality.[85] However, there have been no studies that have examined long-term effectiveness of these agents in reducing chronic complications. In addition, other classes of antidiabetic drugs reduce overall glucose levels, including those in the postprandial period.

Adverse effects of AGIs include flatulence, abdominal discomfort, and diarrhea, frequently leading to discontinuation of the drug. The AGIs are rarely used as monotherapy because of their comparatively mild efficacy. They are approved for use as monotherapy and in combination with SUs. One caveat regarding AGI therapy (specifically, when combined with secretagogues or insulin) is the requirement that hypoglycemia be reversed by consuming glucose itself, as opposed to more complex carbohydrates.

Thiazolidinediones. In 1997, troglitazone, a TZD, was introduced in the United States. It was later removed from the market because of rare idiosyncratic hepatocellular injury.[86] This novel class of drugs, currently represented by rosiglitazone and pioglitazone, has a

Table 2. Antidiabetic Oral Agent Monotherapy: Randomized Head-to-Head Trials*

Source, y	Treatment Arms	Subjects, No.	Study Length	Hemoglobin A_{1c} Results
	Sulfonylureas			
Kitbachi et al,[37] 2000	Glipizide vs glyburide	18	15 mo	Equivalent efficacy
Dills and Schneider,[36] 1996	Glimepiride vs glyburide	577	1 y	Equivalent efficacy
Birkeland et al,[35] 1994	Glipizide vs glyburide	46	15 mo	Equivalent efficacy
Carlson et al,[34] 1993	Glyburide vs micronized glyburide	206	12 wk	Equivalent efficacy
Rosenstock et al,[33] 1993	Glipizide vs glyburide	139	4 mo	Equivalent efficacy
Kilo et al,[32] 1992	Glipizide vs glyburide	34	3 mo	Equivalent efficacy
	Metformin			
Tessier et al,[60] 1999	Metformin vs gliclazide	36	24 wk	Equivalent efficacy
UKPDS,[58] 1998	Meformin vs various SUs	753	10.7 y	Equivalent efficacy
Campbell et al,[61] 1994	Metformin vs glipizide	48	1 y	Metformin more efficacious than glipizide (hemoglobin A_{1c} −2.6% vs −1.9% [P<.05])
Hermann,[62] 1994	Metformin vs glyburide	165	6 mo	Equivalent efficacy
Clarke and Campbell,[63] 1977	Metformin vs chlorpropamide	216	1 y	Equivalent efficacy
	α-Glucosidase Inhibitors			
Hoffmann and Spengler,[57] 1997	Acarbose vs metformin	96	24 wk	Equivalent efficacy, but metformin dose less than maximal at 850 mg twice daily
Segal et al,[83] 1997	Miglitol vs glibenclamide	119	24 wk	Glibenclamide more efficacious than miglitol (hemoglobin A_{1c} −1.0% vs −0.8% [P value not reported]), but mean glibenclamide dose less than maximal at 3.6 mg/d
Hoffmann and Spengler,[82] 1994	Acarbose vs glibenclamide	96	24 wk	Equivalent efficacy, but mean glibenclamide dose less than maximal at 4.3 mg/d
	Thiazolidinediones			
Kirk et al,[103] 1999	Troglitazone vs metformin (in SU-treated patients)	32	14 wk	Equivalent efficacy
Inzucchi et al,[102] 1998	Troglitazone vs metformin	28	3 mo	Equivalent efficacy
Horton et al,[104] 1998	Troglitazone vs glyburide	552	1 y	Equivalent efficacy
	Non-SU Secretagogues			
Horton et al,[134] 2000	Nateglinide vs metformin	701	24 wk	Metformin more effective than nateglinide (−0.3% hemoglobin A_{1c})
Raskin et al,[133] 2000	Repaglinide vs troglitazone	256	22 wk	Repaglinide more effective than troglitazone (hemoglobin A_{1c} −0.8% vs −0.4% [P<.05])
Marbury et al,[129] 1999	Repaglinide vs glyburide	576	12 mo	Equivalent efficacy
Landgraf et al,[130] 1999	Repaglinide vs glibenclamide	195	14 wk	Equivalent efficacy
Wolffenbuttel and Landgraf,[131] 1999	Repaglinide vs glyburide	424	1 y	Equivalent efficacy
Moses et al,[132] 1999	Repaglinide vs metformin	83	3 mo	Equivalent efficacy

*SU indicates sulfonylurea.

unique mechanism of action that remains incompletely understood. Thiazolidinediones are pharmacological ligands for a nuclear receptor known as peroxisome-proliferator-activited receptor gamma. When activated, the receptor binds with response elements on DNA, altering transcription of a variety of genes that regulate carbohydrate and lipid metabolism.[87] The most prominent effect of TZDs is increased insulin-stimulated glucose uptake by skeletal muscle cells.[88-91] Thus, these agents decrease insulin resistance in peripheral tissues. Hepatic glucose production is decreased, although perhaps only at the highest doses.[51,90] Peroxisome-proliferator-activited receptor gamma activation also reduces lipolysis and enhances adipocyte differentiation. It is interesting to consider that the receptor is most highly expressed in adipocytes, while expression in myocytes is comparatively minor. Therefore, the increase in glucose uptake by muscle may largely be an indirect effect mediated through TZD interaction with adipocytes.[92] Candidates for the intermediary signal between fat and muscle include leptin, free fatty acids, tumor necrosis factor α, adiponectin, and the more recently isolated resistin.[93]

As with metformin, the TZDs do not stimulate pancreatic islet cells to secrete more insulin. Indeed, insulin concentrations are usually reduced, perhaps to an even greater extent than with metformin.[56,57] Thiazolidinediones enhance the responsiveness and efficiency of beta cells, presumably by decreasing glucose and free fatty acid levels, both of which have deleterious effects on insulin secretion.[94] Preliminary data also suggest that this drug class may actually prolong beta cell survival.[95,96]

In placebo-controlled trials, TZDs generally lower HbA_{1c} as much as SUs and metformin do,[97-101] and more than AGIs do (Table 1). Head-to-head studies have been performed on TZDs vs metformin[102,103] and SUs,[104] with equivalent reductions in HbA_{1c} (Table 2). No long-term outcome studies on microvascular end points are available. In relatively short-term studies, TZDs appear to lower microalbumin excretion, perhaps a manifestation of their beneficial effect on endothelial function.[100,105] Since TZDs decrease insulin resistance and because the latter is associated with macrovascular disease, some have wondered whether these drugs might provide cardiovascular protection.[38,106,107] Preliminary evidence suggests that this notion may by justified.[108]

In addition to their ability to lower insulin levels, the TZDs also have certain lipid benefits. High-density lipoprotein cholesterol concentrations, for instance, increase with TZD therapy and triglyceride concentrations frequently fall.[101,104] The effect on low-density lipoprotein cholesterol concentrations is more variable, with increases reported with some,[104,109] but not all,[101] agents. Any rise in low-density lipoprotein cholesterol concentrations may be due to a shift from small and dense to large and buoyant low-density lipoprotein particles, which are less atherogenic.[110,111]

Thiazolidinediones also slightly reduce blood pressure,[112] enhance fibrinolysis,[113] and improve endothelial function.[114] These agents also appear to decrease in vitro vascular inflammation and vascular smooth muscle cell proliferation,[115] both important elements in the atherosclerotic process. Animal data suggest an antiatherosclerotic effect.[116] However, whether such nonglycemic effects will eventually translate into benefits on actual clinical end points remains unclear. Only 3 human studies have examined more than just biochemical effects related to vascular disease. In a Japanese investigation troglitazone reduced intimal-medial thickness of carotid arteries in diabetic patients, as measured by ultrasound.[117] Similar changes were more recently reported with pioglitazone by the same group.[118] In another study, troglitazone decreased neointimal proliferation after angioplasty.[119] Several studies examining the clinical implications of these findings are under way, although data most likely will not be available for several more years. The 2 TZDs currently available, rosiglitazone and pioglitazone, appear to have similar efficacy on glycemia.[120]

Adverse effects of TZDs include weight gain, which can be as great or greater than that with the SUs. Weight gain appears to involve mostly peripheral subcutaneous sites, with a reduction in visceral fat depots,[121] the latter being better correlated with insulin resistance. Edema can also occur. Both weight gain and edema are more common in patients who receive TZDs with insulin. Anemia may also occur infrequently. Although the Food and Drug Administration still recommends periodic measurement of hepatic function, the available TZDs, unlike troglitazone, have not been convincingly associated with liver injury. Patients with advanced forms of congestive heart failure and those with hepatic impairment should not receive TZDs. Thiazolidinediones are the most expensive class of antidiabetic medication and are indicated as monotherapy and in combination with metformin, SUs, and insulin (pioglitazone only).

Non-SU Secretagogues. The mechanism of action of the non-SU insulin secretagogues (repaglinide [a benzoic acid derivative] and nateglinide [a phenylalanine derivative]) is similar to that of SUs: interaction with voltage-dependent K_{ATP} channels on beta cells. They are distinguished from the SUs by their short metabolic half-lives, which result in brief episodic stimulation of insulin secretion.[122] There are 2 important consequences from this difference. First, postprandial glucose excursions are attenuated because of greater insulin secretion immediately after meal ingestion.[123] Second, because less insulin is secreted several hours after the meal, there is decreased risk of hypoglycemia during this late postprandial phase.[124] One agent, nateglinide, has little stimulatory effect on insulin secretion when administered in the fasting state.[125] Thus, nateglinide may enhance meal-stimulated insulin secretion more than other secretagogues do. Efficacy of repaglinide is similar to that of SUs,[126,127]

whereas nateglinide appears to be somewhat less potent a secretagogue (Table 1).[128] Three comparative trials[129-131] (Table 2) of repaglinide vs SU have been published, each showing equal lowering of glucose levels. In single studies, the efficacy of repaglinide was equal to that of metformin[132] but greater than that of troglitazone.[133] In 1 study, nateglinide was less efficacious than metformin.[134] These drugs have not been assessed for their long-term effectiveness in decreasing microvascular or macrovascular risk. Adverse effects include hypoglycemia and weight gain, which are probably less pronounced than that caused by the SUs.[129] One disadvantage of this drug category is the frequent dosing schedule required with meals. Repaglinide and nateglinide are hepatically metabolized and renally cleared and should be use cautiously when function of the liver and kidneys is impaired. They are approved for use either as monotherapy or in combination with metformin.

Monotherapy Recommendations

Given the myriad therapeutic options available for type 2 DM, how does the physician choose the best drug for a specific patient? Except for the AGIs and nateglinide, which are generally less effective, each of the other drugs will lead to a similar reduction in HbA_{1c}. TABLE 3 summarizes the relative advantages and disadvantages of different drug classes. Does one drug class hold an advantage over the others? Because metformin is the only drug associated with weight loss, or at least weight neutrality, it has become the most widely prescribed single antihyperglycemic drug and is generally regarded as the best first-line agent, at least in the obese patient without contraindications for its use. Its favorable performance in the UKPDS[58] supports this approach. In addition, the virtual lack of hypoglycemia makes metformin therapy an attractive option, particularly in patients whose control is approaching the euglycemic range.

The precise role for the insulin secretagogues is evolving. Their association with hypoglycemia and weight gain remains problematic, and concerns regarding hyperinsulinemia and beta cell exhaustion persist. Although cost-effective in terms of glucose lowering effect, these agents are being used less as first-line therapy. They remain an important element of combination regimens. Even in an era with increasing emphasis on the role of insulin resistance in type 2 DM, insulin deficiency remains a critical pathophysiological

Table 3. Currently Available Oral Therapeutic Options for Type 2 Diabetes Mellitus

Sulfonylureas (SUs)	Non-SU Secretagogues	Biguanides	α-Glucosidase Inhibitors	Thiazolidinediones
Mechanism of action				
Increased pancreatic insulin secretion	Increased pancreatic insulin secretion	Decreased hepatic glucose production	Decreased gut carbohydrate absorption	Increased peripheral glucose disposal
Advantages				
Well established	Targets postprandial glycemia	Well established	Targets postprandial glycemia	No hypoglycemia
Decreases microvascular risk	Possibly less hypoglycemia and weight gain than with SUs	Weight loss	No hypoglycemia	Reverses prime defect of type 2 diabetes
Convenient daily dosing		No hypoglycemia	Nonsystemic	Nonglycemic benefits (decreased lipid levels, increased fibrinolysis, decreased hyperinsulinemia, improved endothelial function)
		Decreases microvascular risk		Possible beta cell preservation
		Decreases macrovascular risk		Convenient daily dosing
		Nonglycemic benefits (decreased lipid levels, increased fibrinolysis, decreased hyperinsulinemia)		
		Convenient daily dosing		
Disadvantages				
Hypoglycemia	More complex (3 times daily) dosing schedule	Adverse gastrointestinal effects	More complex (3 times daily) dosing schedule	Liver function test monitoring
Weight gain	Hypoglycemia	Many contraindications	Adverse gastrointestinal effects	Weight gain
Hyperinsulinemia (role uncertain)	Weight gain	Lactic acidosis (rare)	No long-term data	Edema
	No long-term data			Slow onset of action
	Hyperinsulinemia (role uncertain)			No long-term data
Food and Drug Administration approval status				
Monotherapy	Monotherapy	Monotherapy	Monotherapy	Monotherapy
Combination with insulin, metformin, thiazolidinedione, α-glucosidase inhibitors	Combination with metformin	Combination with insulin, SU, non-SU secretagogue, thiazolidinedione	Combination with SU	Combination with insulin (pioglitazone only), SU, metformin

target of therapy.[135,136] In addition, some consider them the best first-line agents in nonobese patients who have type 2 DM and may exhibit more pancreatic secretory dysfunction than insulin resistance.[137] Although metformin's benefits in reducing cardiovascular end points were demonstrated solely in overweight patients in the UKPDS, its effect on lowering glucose levels is not predicted by body weight.[52,62] (In general, the predictors of response to other drug classes have not been well studied. In a meta-analysis, the best responders to TZD therapy had the highest C-peptide concentrations, suggesting greater insulin resistance, more preserved beta cell function, or both.)[138]

The niche for the non-SU secretagogues is also unclear. They may be preferred in those who require secretagogue therapy and have irregular meal schedules. However, their use must be balanced with their increased cost compared with the now generic SUs and a considerably less convenient dosing schedule.

Although the TZDs are interesting compounds of great promise, there are no long-term data on microvascular or macrovascular risk. One may presume that any agent that lowers glucose levels will eventually lead to a similar microvascular risk reduction as other agents. Indeed, long-term studies of the impact of the newer agents on microvascular end points are unlikely, given the now accepted benefit of conventional agents in this regard. An effect on macrovascular disease is more difficult to predict because of the lack of convincing data that this disease is necessarily associated solely with glucose-level control. Evidence is mounting for an antiatherosclerotic potential for the TZDs,[39,106,107] but because of their increased cost, the continued requirement for liver function test monitoring, and the potential for weight gain and edema, they are not widely considered the ideal monotherapy choice. Somewhat paradoxically, TZDs appear to be most effective when used with the earliest forms of diabetes, such as in the drug-naive patient,[98,101] when insulin secretion is still substantial. As more data emerge regarding beta cell preservation,[94,95] which may be a unique benefit of this class, and cardiovascular risk reduction, the TZDs or similar drugs may one day emerge as the best first-line agent for diabetes. However unlike other agents, TZDs may take weeks or sometimes months to exert their full glycemic effect. Therefore, they are less attractive when rapid lowering of glucose levels is desired.

In summary, in terms of antihyperglycemic effect alone, there is no compelling reason to favor one of the major categories of antidiabetic agents (SUs, biguanides, and TZDs) over another. However, metformin's performance in the UKPDS in obese patients, ie, its lack of associated hypoglycemia and weight gain, make it the most attractive option for obese—if not all—patients who have type 2 DM but no contradindications for its use. The emerging TZD class may provide for additional cardiovascular protection for type 2 DM patients, but TZDs' cost and adverse-effect profile make them less fitting as monotherapy, unless metformin is contraindicated or poorly tolerated. The actual choice of a drug, however, must be based on a variety of clinical factors and individual patient characteristics, including predisposition to adverse effects, the degree of hyperglycemia, and cost. The paramount concern of the physician should be attainment of the best glycemic control with whatever antidiabetic regimen is well tolerated.

Combination Therapy

Given the multiple pathophysiological lesions in type 2 DM, combination therapy is a logical approach to its management. The UKPDS clearly demonstrated that type 2 DM is a progressive disease. After 3 years, for example, type 2 DM in only 50% of patients was adequately controlled with a single drug, and after 9 years, this percentage had decreased to 25%.[139]

Each clinical trial that has examined the addition of an oral agent to that of another class has demonstrated additive HbA_{1c} reduction (TABLE 4).[104,132,134,140-151] With few exceptions,[104] the effect on HbA_{1c} has been similar to the effect from using the added drug as monotherapy vs placebo. The most popular combinations are SU and metformin, metformin and TZD, and SU and TZD. Triple combination therapy, typically SU, metformin, and TZD, improved glycemia in 1 placebo-controlled study[152] but is not formally approved by the Food and Drug Administration. Since HbA_{1c} reduction is the overriding goal in all patients, the precise combination used may not be as important as the glucose levels achieved. There is no evidence that a specific combination is any more effective in lowering glucose levels or more effective in preventing complications than another. So the same patient-specific criteria that go into the decision tree for monotherapy apply when more complex regimens are constructed. In the UKPDS, however, a group of patients who did not achieve acceptable control with SU therapy was randomized to the early addition of metformin. The results of this substudy were somewhat unexpected in that combination therapy was associated with a 96% increase in diabetes-related mortality.[58] The authors performed an epidemiological analysis on all subjects who received this combination, and overall, no increased risk was shown. Because a deleterious effect of such a commonly used combination is not biologically plausible, avoiding the combination is not recommended. In fact, a fixed combination tablet containing glyburide and metformin has recently become available. Combination therapy involving 2 or 3 drug classes with distinct mechanisms of action will not only improve glycemic control, but also result in lower overall drug dosing in some settings[140] and minimize adverse effects. If glycemic control cannot be attained with oral agents alone, there should be no hesitation about using insulin either alone[152] or in combination[153,154] with oral agents. The latter approach may be preferred, since it leads to im-

proved glycemic control and a lower insulin dose compared with insulin monotherapy.

COMMENT

Type 2 diabetes mellitus is a complex disorder associated with significant health and economic burdens. Keeping blood glucose levels near the normal range lowers the risk of complications and is an important therapeutic goal. A number of oral antihyperglycemic agents have been introduced in the United States during the past several years, each with its own mode of action. They are equally effective in lowering blood glucose concentrations and HbA_{1c}, except for the AGIs and nateglinide, which in general appear to be less potent. Only SUs and metformin have been shown to reduce microvascular complications, with metformin exhibiting additional benefits on macrovascular risk. Because they lower glucose levels, however, the remaining drug classes will presumably have a proportionate effect on microvascular complications. Their effect on macrovascular risk, however, remains unkown. The proper choice of antidiabetic agent for a patient is based on several factors. Each drug class has unique adverse effects and prescribing precautions. The popularity of insulin sensitizers, defined broadly as metformin and TZDs, is increasing, since these agents avoid the risk of hypoglycemia associated with secretagogue therapy and allow for the treatment of patients already near the euglycemic range. Most patients will require combination therapy as their disease progresses.

Table 4. Antidiabetic Oral Agent Combination Therapy: Randomized Controlled Trials*

Source, y	Randomization	Subject No.	Study Length	Hemoglobin A_{1c} Reduction, %†
Erle et al,[140] 1999	Glyburide + metformin vs glyburide + placebo	40	6 mo	1.0
UKPDS,[58] 1998	SU + metformin vs SU alone	591	3 y	0.6
DeFronzo and Goodman,[52] 1995	Glyburide + metformin vs glyburide alone	632	29 wk	1.6
Standl et al,[146] 2001	Metformin/glyburide + miglitol vs metformin/glyburide + placebo	154	24 wk	0.4
Willms and Ruge,[145] 1999	SU + acarbose vs SU + metformin vs SU + placebo	89	12 wk	1.0 (+ acarbose), 1.2 (+ metformin)
Holman et al,[144] 1999	Variety of treatments + acarbose vs variety of treatments + placebo	973	3 y	0.2
Rosenstock et al,[142] 1998	Metformin + acarbose vs metformin + placebo	148	24 wk	0.7
Scorpiglione et al,[143] 1999	Variety of treatments + acarbose vs variety of treatments + placebo	250	12 mo	0.1 (P = NS)
Johnston et al,[84] 1994	SU + miglitol vs SU + placebo	192	14 wk	0.8
Costa and Pinol,[141] 1997	Glibenclamide + acarbose vs glibenclamide + placebo	65	6 mo	0.8
Coniff et al,[81] 1995	Tolbutamide + acarbose vs either drug alone	290	24 wk	0.4 (vs tolbutamide), 0.8 (vs acarbose) Note: acarbose dose 200 mg 3 times daily (above Food and Drug Administration maximum)
Chiasson et al,[74] 1994	Metformin or SU + acarbose vs metformin or SU + placebo	354	1 y	0.8 to 0.9
Yale et al,[152] 2001	Metformin + SU + troglitazone vs metformin + SU + placebo	200	1 y	1.4
Einhorn et al,[150] 2000	Metformin + pioglitazone vs metformin + placebo	328	16 wk	0.8
Fonseca et al,[109] 2000	Metformin + rosiglitazone vs metformin + placebo	348	26 wk	1.2
Wolffenbuttel et al,[151] 2000	SU + rosiglitazone vs SU + placebo	574	26 wk	1.0
Buysschaert et al,[148] 1999	SU + troglitazone vs SU + placebo	259	16 wk	0.2, but troglitazone dose only 200 mg/d
Horton et al,[104] 1998	Glyburide + troglitazone vs either drug alone	552	1 y	2.7
Iwamoto et al,[147] 1996	SU + troglitazone vs SU + placebo	291	12 wk	0.9
Raskin et al,[133] 2000	Troglitazone + repaglinide vs either drug alone	256	22 wk	1.3 vs troglitazone, 0.9 vs repaglinide
Moses et al,[132] 1999	Metformin + repaglinide vs either drug alone	83	3 mo	1.1 vs metformin, 1.0 vs repaglinide
Horton et al,[134] 2000	Metformin + nateglinide vs either drug alone	701	24 wk	0.6 vs metformin, 0.9 vs nateglinide

*SU indicates sulfonylurea.
†Unless otherwise indicated, values represent the absolute percent reduction in hemoglobin A_{1c} of combination therapy vs monotherapy.

REFERENCES

1. Harris MI. Diabetes in America: epidemiology and scope of the problem. *Diabetes Care*. 1998;21(suppl 3):C11-C14.
2. Lindeman RD, Romero LJ, Hundley R, et al. Prevalences of type 2 diabetes, the insulin resistance syndrome, and coronary heart disease in an elderly, biethnic population. *Diabetes Care*. 1998;21:959-966.
3. Rosenbloom AL, Joe JR, Young RS, Winter WE. Emerging epidemic of type 2 diabetes in youth. *Diabetes Care*. 1999;22:345-354.
4. Herman WH, Eastman RC. The effects of treatment on the direct costs of diabetes. *Diabetes Care*. 1998;21(suppl 3):C19-C24.
5. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329:977-986.
6. Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract*. 1995;28:103-117.
7. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998;352:837-853.
8. Ferrannini E. Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. *Endocr Rev*. 1998;19:477-490.
9. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med*. 1993;329:1988-1992.
10. Weyer C, Hanson RL, Tataranni PA, Bogardus C, Pratley RE. A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: evidence for a pathogenic role of relative hyperinsulinemia. *Diabetes*. 2000;49:2094-2101.
11. Perseghin G, Ghosh S, Gerow K, Shulman GI. Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes*. 1997;46:1001-1009.
12. Ishikawa M, Pruneda ML, Adams-Huet B, Raskin P. Obesity-independent hyperinsulinemia in nondiabetic first-degree relatives of individuals with type 2 diabetes. *Diabetes*. 1998;47:788-792.
13. Axelsen M, Smith U, Eriksson JW, Taskinen MR, Jansson PA. Postprandial hypertriglyceridemia and insulin resistance in normoglycemic first-degree relatives of patients with type 2 diabetes. *Ann Intern Med*. 1999;131:27-31.
14. Stern M. Natural history of macrovascular disease in type 2 diabetes: role of insulin resistance. *Diabetes Care*. 1999;22(suppl 3):C2-C5.
15. Fagan TC, Deedwania PC. The cardiovascular dysmetabolic syndrome. *Am J Med*. 1998;105:77S-82S.
16. Yki-Jarvinen H. Acute and chronic effects of hyperglycaemia on glucose metabolism: implications for the development of new therapies. *Diabet Med*. 1997;14(suppl 3):S32-S37.
17. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*. 1963;1:785-789.
18. Roden M, Price TB, Perseghin G, et al. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest*. 1996;97:2859-2865.
19. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocr Metab*. 2000;11:351-356.
20. American Diabetes Association. Standards of medical care for patients with diabetes mellitus. *Diabetes Care*. 2001;24(suppl 1):S33-S43.
21. ACE Consensus Conference on Guidelines for Glycemic Control. *Endocr Pract*. In press.
22. American Diabetes Association. Translation of the diabetes nutrition recommendations for health care institutions. *Diabetes Care*. 1997;20:106-108.
23. Tinker LF, Heins JM, Holler HJ. Commentary and translation: 1994 nutrition recommendations for diabetes: Diabetes Care and Education, a practice group of the American Dietetic Association. *J Am Diet Assoc*. 1994;94:507-511.
24. Cohen KL, Harris S. Efficacy of glyburide in diabetics poorly controlled on first-generation oral hypoglycemics. *Diabetes Care*. 1987;10:555-557.
25. Zimmerman BR. Sulfonylureas. *Endocrinol Metab Clin North Am*. 1997;26:511-521.
26. Doar JW, Thompson ME, Wilde CE, Sewell PF. Diet and oral antidiabetic drugs and plasma sugar and insulin levels in patients with maturity-onset diabetes mellitus. *BMJ*. 1976;1:498-500.
27. Kolterman OG, Prince MJ, Olefsky JM. Insulin resistance in noninsulin-dependent diabetes mellitus: impact of sulfonylurea agents in vivo and in vitro. *Am J Med*. 1983;74:82-101.
28. Simonson DC, Ferrannini E, Bevilacqua S, et al. Mechanism of improvement in glucose metabolism after chronic glyburide therapy. *Diabetes*. 1984;33:838-845.
29. Schade DS, Jovanovic L, Schneider J. A placebo-controlled, randomized study of glimepiride in patients with type 2 diabetes mellitus for whom diet therapy is unsuccessful. *J Clin Pharmacol*. 1998;38:636-641.
30. Simonson DC, Kourides IA, Feinglos M, Shamoon H, Fischette CT. Efficacy, safety, and dose-response characteristics of glipizide gastrointestinal therapeutic system on glycemic control and insulin secretion in NIDDM: results of two multicenter, randomized, placebo-controlled clinical trials: the Glipized Gastrointestinal Therapeutic System Study Group. *Diabetes Care*. 1997;20:597-606.
31. Rosenstock J, Samols E, Muchmore DB, Schneider. Glimepiride, a new once-daily sulfonylurea: a double-blind placebo-controlled study of NIDDM patients. *Diabetes Care*. 1996;19:1194-1199.
32. Kilo C, Meenan A, Bloomgaren Z. Glyburide versus glipizide in the treatment of patients with non-insulin-dependent diabetes mellitus. *Clin Ther*. 1992;14:801-812.
33. Rosenstock J, Corrao PJ, Goldberg RB, Kilo C. Diabetes control in the elderly: a randomized, comparative study of glyburide versus glipizide in non-insulin-dependent diabetes mellitus. *Clin Ther*. 1993;15:1031-1040.
34. Carlson RF, Isley WL, Ogrinc FG, Klobucar TR. Efficacy and safety of reformulated, micronized glyburide tablets in patients with non-insulin-dependent diabetes mellitus: a multicenter, double-blind, randomized trial. *Clin Ther*. 1993;15:788-796.
35. Birkeland KI, Furuseth K, Melander A, Mowinckel P, Vaaler S. Long-term randomized placebo-controlled double-blind therapeutic comparison of glipizide and glyburide: glycemic control and insulin secretion during 15 months. *Diabetes Care*. 1994;17:45-49.
36. Dills DG, Schneider J. Clinical evaluation of glimepiride versus glipizide in NIDDM in a double-blind comparative study: Glimepiride/Glyburide Research Group. *Horm Metab Res*. 1996;28:426-429.
37. Kitbachi AE, Kaminska E, Fisher JN, et al. Comparative efficacy and potency of long-term therapy with glipizide or glyburide in patients with type 2 diabetes mellitus. *Am J Med Sci*. 2000;319:143-148.
38. Klepzig H, Kober G, Matter C, et al. Sulfonylureas and ischaemic preconditioning: a double-blind, placebo-controlled evaluation of glimepiride and glibenclamide. *Eur Heart J*. 1999;20:403-405.
39. Henry RR. Type 2 diabetes care: the role of insulin-sensitizing agents and practical implications for cardiovascular disease prevention. *Am J Med*. 1998;105:20S-26S.
40. Garratt KN, Brady PA, Hassinger NL, Grill DE, Terzic A, Holmes DR Jr. Sulfonylurea drugs increase early mortality in patients with diabetes mellitus after direct angioplasty for acute myocardial infarction. *J Am Coll Cardiol*. 1999;33:119-124.
41. Goldner MG, Knatterud GL, Prout TE. Effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes, III: clinical implications of UGDP results. *JAMA*. 1971;218:1400-1410.
42. Kilo C, Miller JP, Williamson JR. The crux of the UGDP: spurious results and biologically inappropriate data analysis. *Diabetologia*. 1980;18:179-185.
43. Stenman S, Melander A, Groop PH, Groop LC. What is the benefit of increasing the sulfonylurea dose? *Ann Intern Med*. 1993;118:169-172.
44. Bailey CJ, Turner RC. Metformin. *N Engl J Med*. 1996;334:574-579.
45. Kolata GB. The phenformin ban: is the drug an imminent hazard? *Science*. 1979;203:1094-1096.
46. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1995;333:550-554.
47. Johansen K. Efficacy of metformin in the treatment of NIDDM: meta-analysis. *Diabetes Care*. 1999;22:33-37.
48. Inzucchi SE, Maggs DG, Spollett GR, et al. Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med*. 1998;338:867-872.
49. Hundal RS, Krssak M, Dufour S, et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes*. 2000;49:2063-2069.
50. Cusi K, DeFronzo RA. Metformin: a review of its metabolic effects. *Diabet Rev*. 1998;6:89-130.
51. Yu JG, Kruszynska YT, Mulford MI, Olefsky JM. A comparison of troglitazone and metformin on insulin requirements in euglycemic intensively insulin-treated type 2 diabetic patients. *Diabetes*. 1999;48:2414-2421.
52. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus: the Multicenter Metformin Study Group. *N Engl J Med*. 1995;333:541-549.
53. Dornan T, Heller S, Peck G, Tattersall R. Double-blind evaluation of efficacy and tolerability of metformin in NIDDM. *Diabetes Care*. 1991;14:342-344.
54. Nagi D, Yudkin J. Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects: a study of two ethnic groups. *Diabetes Care*. 1993;16:621-629.
55. Grant PJ. The effects of high- and medium-dose metformin therapy on cardiovascular risk factors in patients with type II diabetes. *Diabetes Care*. 1996;19:64-66.
56. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. *Am J Med*. 1997;103:491-497.
57. Hoffmann J, Spengler M. Efficacy of 24-week monotherapy with acarbose, metformin, or placebo in dietary-treated NIDDM patients: the Essen-II Study. *Am J Med*. 1997;103:483-490.
58. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet*. 1998;352:854-865.

59. Johansen K. Efficacy of metformin in the treatment of NIDDM. *Diabetes Care*. 1999;22:33-37.
60. Tessier D, Maheux P, Khalil A, Fulop T. Effects of gliclazide versus metformin on the clinical profile and lipid peroxidation markers in type 2 diabetes. *Metab Clin Exp*. 1999;48:897-903.
61. Campbell IW, Menzies DG, Chalmers J, McBain AM, Brown IR. One year comparative trial of metformin and glipizide in type 2 diabetes mellitus. *Diabetes Metab*. 1994;20:394-400.
62. Hermann LS. Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations: a double-blind controlled study. *Diabetes Care*. 1994;17:1100-1109.
63. Clarke BF, Campbell IW. Comparison of metformin and chlorpropamide in non-obese, maturity-onset diabetics uncontrolled by diet. *BMJ*. 1977;2:1576-1578.
64. Fontbonne A, Charles MA, Juhan-Vague I, et al. The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution: BIGPRO Study Group. *Diabetes Care*. 1996;19:920-926.
65. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. *J Am Coll Cardiol*. 2001;37:1344-1350.
66. Misbin RI, Green L, Stadel BV, Gueriguian JL, Gubbi A, Fleming GA. Lactic acidosis in patients with diabetes treated with metformin. *N Engl J Med*. 1998;338:265-266.
67. Turner RC. The U.K. Prospective Diabetes Study: a review. *Diabetes Care*. 1998;21(suppl 3):C35-C38.
68. Goke B, Herrmann-Rinke C. The evolving role of alpha-glucosidase inhibitors. *Diabetes Metab Res Rev*. 1998;14(suppl 1):S31-S38.
69. Lebowitz HE. α-Glucosidase inhibitors as agents in the treatment of diabetes. *Diabetes Rev*. 1998;6:132-145.
70. Hanefeld M, Fischer S, Schulze J, et al. Therapeutic potentials of acarbose as first-line drug in NIDDM insufficiently treated with diet alone. *Diabetes Care*. 1991;14:732-737.
71. Hotta N, Kabuta H, Sano T, et al. Long-term effect of acarbose on glycaemic control in non-insulin-dependent diabetes mellitus: a placebo-controlled double-blind study. *Diabetes Med*. 1993;10:134-138.
72. Santeusanio F, Ventura MM, Contandini S, Compagnucci P, Moriconni V, Zaccarini P. Efficacy and safety of two different doses of acarbose in non-insulin-dependent diabetic patients treated by diet alone. *Diabetes Nutr Metab*. 1993;6:147-154.
73. Coniff RF, Shapiro JA, Seaton TB. Long-term efficacy and safety of acarbose in the treatment of obese subjects with non-insulin-dependent diabetes mellitus. *Arch Intern Med*. 1994;154:2442-2448.
74. Chiasson JL, Josse RG, Hunt JA, et al. The efficacy of acarbose in the treatment of patients with non-insulin-dependent diabetes mellitus: a multicenter controlled clinical trial. *Ann Intern Med*. 1994;121:928-935.
75. Coniff RF, Shapiro JA, Robbins D, et al. Reduction of glycosylated hemoglobin and postprandial hyperglycemia by acarbose in patients with NIDDM: a placebo-controlled dose-comparison study. *Diabetes Care*. 1995;18:817-824.
76. Braun D, Schonherr U, Mitzkat H-J. Efficacy of acarbose monotherapy in patients with type 2 diabetes: a double-blind study conducted in general practice. *Endocrinol Metab*. 1996;3:275-280.
77. Fischer S, Hanefeld M, Spengler M, Boehme K, Temelkova-Kurktschiev T. European study on dose-response relationship of acarbose as a first-line drug in non-insulin-dependent diabetes mellitus: efficacy and safety of low and high doses. *Acta Diabetol*. 1998;35:34-40.
78. Johnston PS, Feig PU, Coniff RF, Krol A, Kelley DE, Mooradian AD. Chronic treatment of African-American type 2 diabetic patients with alpha-glucosidase inhibition. *Diabetes Care*. 1998;21:416-422.
79. Scott R, Lintott CJ, Zimmet Campbell L, Bowen K, Welborn T. Will acarbose improve the metabolic abnormalities of insulin-resistant type 2 diabetes mellitus? *Diabetes Res Clin Pract*. 1999;43:179-185.
80. Hasche H, Mertes G, Bruns C, et al. Effects of acarbose treatment in Type 2 diabetic patients under dietary training: a multicentre, double-blind, placebo-controlled, 2-year study. *Diabetes Nutr Metab*. 1999;12:277-285.
81. Coniff RF, Shapiro JA, Seaton TB, Bray GA. Multicenter, placebo-controlled trial comparing acarbose with placebo, tolbutamide, and tolbutamide-plus-acarbose in non-insulin-dependent diabetes mellitus. *Am J Med*. 1995;98:443-451.
82. Hoffmann J, Spengler M. Efficacy of 24-week monotherapy with acarbose, glibenclamide, or placebo in NIDDM patients: the Essen Study. *Diabetes Care*. 1994;17:561-566.
83. Segal P, Feig PU, Schernthaner G, et al. The efficacy and safety of miglitol therapy compared with glibenclamide in patients with NIDDM inadequately controlled by diet alone. *Diabetes Care*. 1997;20:687-691.
84. Johnston PS, Coniff RF, Hoogwerf BJ, Santiago JV, Pi-Sunyer FX, Krol A. Effects of the carbohydrase inhibitor miglitol in sulfonylurea-treated NIDDM patients. *Diabetes Care*. 1994;17:20-29.
85. Donahue RP, Abbott RD, Reed DM, Yano K. Postchallenge glucose concentration and coronary heart disease in men of Japanese ancestry: Honolulu Heart Program. *Diabetes*. 1987;36:689-692.
86. Murphy EJ, Davern TJ, Shakil AO, et al. Troglitazone-induced fulminant hepatic failure: Acute Liver Failure Study Group. *Dig Dis Sci*. 2000;45:549-553.
87. Mudaliar S, Henry RR. New oral therapies for type 2 diabetes mellitus: the glitazones or insulin sensitizers. *Annu Rev Med*. 2001;52:239-257.
88. Frias JP, Yu JG, Kruszynska YT, Olefsky JM. Metabolic effects of troglitazone therapy in type 2 diabetic, obese, and lean normal subjects. *Diabetes Care*. 2000;23:64-69.
89. Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Engl J Med*. 1994;331:1188-1193.
90. Maggs DG, Buchanan TA, Burant CF, et al. Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus. *Ann Intern Med*. 1998;128:176-185.
91. Petersen KF, Krssak M, Inzucchi S, Cline GW, Dufour S, Shulman GI. Mechanism of troglitazone action in type 2 diabetes. *Diabetes*. 2000;49:827-831.
92. Chao L, Marcus-Samuels B, Mason MM, et al. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest*. 2000;106:1221-1228.
93. Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. *Nature*. 2001;409:307-312.
94. Cavaghan MK, Ehrmann DA, Byrne MM, Polonsky KS. Treatment with the oral antidiabetic agent troglitazone improves beta cell responses to glucose in subjects with impaired glucose tolerance. *J Clin Invest*. 1997;100:530-537.
95. Buchanan TA, Xiang AH, Peters RK, et al. Response of pancreatic beta-cells to improved insulin sensitivity in women at high risk for type 2 diabetes. *Diabetes*. 2000;49:782-788.
96. Finegood DT, McArthur MD, Kojwang D, et al. Beta-cell mass dynamics in Zucker diabetic fatty rats: rosiglitazone prevents the rise in net cell death. *Diabetes*. 2001;50:1021-1029.
97. Kumar S, Boulton AJM, Beck-Nielsen H. Troglitazone, an insulin action enhancer, improves metabolic control in NIDDM patients. *Diabetologia*. 1996;39:701-709.
98. Fonseca VA, Valiquett TR, Huang SM, Ghazzi MN, Whitcomb RW. Troglitazone monotherapy improves glycemic control in patients with type 2 diabetes mellitus: a randomized, controlled study: the Troglitazone Study Group. *J Clin Endocrinol Metab*. 1998;83:3169-3176.
99. Phillips LS, Grunberger G, Miller E, Patwardhan R, Rappaport EB, Salzman A. The Rosiglitazone Clinical Trials Study Group: once- and twice-dosing with rosiglitazone improves glycemic control in patients with type 2 diabetes. *Diabetes Care*. 2001;24:308-315.
100. Lebovitz HE, Dole JF, Patwardhan R, Rappaport EB, Freed MI. The Rosiglitazone Clinical Trials Study Group: rosiglitazone monotherapy is effective in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2001;86:280-288.
101. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study: the Pioglitazone 001 Study Group. *Diabetes Care*. 2000;23:1605-1611.
102. Inzucchi SE, Maggs DG, Spollett GR, et al. Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med*. 1998;338:867-872.
103. Kirk JK, Pearce KA, Michielutte R, Summerson JH. Troglitazone or metformin in combination with sulfonylureas for patients with type 2 diabetes? *J Fam Pract*. 1999;48:879-882.
104. Horton ES, Whitehouse F, Ghazzi MN, Venable TC, Whitcomb RW. Troglitazone in combination with sulfonylureas restores glycemic control in patients with type 2 diabetes: the Troglitazone Study Group. *Diabetes Care*. 1998;21:1462-1469.
105. Imano E, Kanda T, Nakatani Y, et al. Effect of troglitazone on microalbuminuria in patients with incipient diabetic nephropathy. *Diabetes Care*. 1998;21:2135-2139.
106. Ginsberg H, Plutsky J, Sobel BE. A review of metabolic and cardiovascular effects of oral antidiabetic agents: beyond glucose-level lowering. *J Cardiovasc Risk*. 1999;6:337-346.
107. Campbell IW. Antidiabetic drugs present and future: will improving insulin resistance benefit cardiovascular risk in type 2 diabetes mellitus? *Drugs*. 2000;60:1017-1028.
108. Parulkar AA, Pendergrass ML, Granda-Ayala R, Lee TR, Fonseca VA. Nonhypoglycemic effects of thiazolidinediones. *Ann Intern Med*. 2001;134:61-71.
109. Fonseca V, Rosenstock J, Patwardhan R, Salzman A. Effect of metformin and rosiglitazone combination therapy in patients with type 2 diabetes mellitus: a randomized controlled trial. *JAMA*. 2000;283:1695-1702.
110. Tack CJ, Smits P, Demacker PN, Stalenhoef AF. Troglitazone decreases the proportion of small, dense LDL and increases the resistance of LDL to oxidation in obese subjects. *Diabetes Care*. 1998;21:796-799.
111. Sunayama S, Watanabe Y, Ohmura H, et al. Effects of troglitazone on atherogenic lipoprotein phenotype in coronary patients with insulin resistance. *Atherosclerosis*. 1999;146:187-193.
112. Sung BH, Izzo JL Jr, Dandona P, Wilson MF. Vasodilatory effects of troglitazone improve blood pressure at rest and during mental stress in type 2 diabetes mellitus. *Hypertension*. 1999;34:83-88.
113. Kruszynska YT, Yu JG, Olefsky JM, Sobel BE. Effects of troglitazone on blood concentrations of plasminogen activator inhibitor 1 in patients with type 2 diabetes and in lean and obese normal subjects. *Diabetes*. 2000;49:633-639.
114. Kotchen TA, Zhang HY, Reddy S, Hoffmann RG. Effect of pioglitazone on vascular reactivity in vivo and in vitro. *Am J Physiol*. 1996;270(suppl 3, pt 2):R660-R666.
115. Hsueh WA, Jackson S, Law RE. Control of vascular cell proliferation and migration by PPAR-gamma:

a new approach to the macrovascular complications of diabetes. *Diabetes Care*. 2001;24:392-397.
116. Collins AR, Meehan WP, Kintscher U, et al. Troglitazone inhibits formation of early atherosclerotic lesions in diabetic and nondiabetic low density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2001;21:365-371.
117. Minamikawa J, Tanaka S, Yamauchi M, Inoue D, Koshiyama H. Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes.
J Clin Endocrinol Metab. 1998;83:1818-1820.
118. Koshiyama H, Shimono D, Kuwamura N, Minamikawa J, Nakamura Y. Rapid communication: inhibitory effect of pioglitazone on carotid arterial wall thickness in type 2 diabetes. *J Clin Endocrinol Metab*. 2001;86:3452-3456.
119. Takagi T, Akasaka T, Yamamuro A, et al. Troglitazone reduces neointimal tissue proliferation after coronary stent implantation in patients with non-insulin dependent diabetes mellitus: a serial intravascular ultrasound study. *J Am Coll Cardiol*. 2000;36:1529-1535.
120. Gegich CG, Altheimer MD. Comparison of effects of thiazolidinediones on cardiovascular risk factors: observations from a clinical practice. *Endocr Pract*. 2001;7:162-169.
121. Kelly IE, Han TS, Walsh K, Lean ME. Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. *Diabetes Care*. 1999;22:288-293.
122. Perfetti R, Ahmad A. Novel sulfonylurea and non-sulfonylurea drugs to promote the secretion of insulin. *Trends Endocrinol Metab*. 2000;11:218-223.
123. Hirschberg Y, Karara AH, Pietri AO, McLeod JF. Improved control of mealtime glucose excursions with coadministration of nateglinide and metformin. *Diabetes Care*. 2000;23:349-353.
124. Nattrass M, Lauritzen T. Review of prandial glucose regulation with repaglinide: a solution to the problem of hypoglycaemia in the treatment of type 2 diabetes? *Int J Obes Relat Metab Disord*. 2000;24 (suppl 3):S21-S31.
125. Keilson L, Mather S, Walter YH, Subramanian S, McLeod JF. Synergistic effects of nateglinide and meal administration on insulin secretion in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2000; 85:1081-1086.
126. Jovanovic L, Dailey G III, Huang WC, Strange P, Goldstein BJ. Repaglinide in type 2 diabetes: a 24-week, fixed-dose efficacy and safety study. *J Clin Pharmacol*. 2000;40:49-57.
127. Goldberg RB, Einhorn D, Lucas CP, et al. A randomized placebo-controlled trial of repaglinide in the treatment of type 2 diabetes. *Diabetes Care*. 1998; 21:1897-1903.
128. Hanefeld M, Bouter KP, Dickinson S, Guitard C. Rapid and short-acting mealtime insulin secretion with nateglinide controls both prandial and mean glycemia. *Diabetes Care*. 2000;23:202-207.
129. Marbury T, Huang WC, Strange P, Lebowitz H. Repaglinide versus glyburide: a one-year comparison trial. *Diabetes Res Clin Pract*. 1999;43:155-166.
130. Landgraf R, Bilo HJ, Muller PG. A comparison of repaglinide and glibenclamide in the treatment of type 2 diabetic patients previously treated with sulphonylureas. *Eur J Clin Pharm*. 1999;55:165-171.
131. Wolffenbuttel BH, Landgraf R. A 1-year multicenter randomized double-blind comparison of repaglinide and glyburide for the treatment of type 2 diabetes: Dutch and German Repaglinide Study Group. *Diabetes Care*. 1999:22:463-477.
132. Moses R, Slobodniuk R, Boyages S, et al. Effect of repaglinide addition to metformin monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care*. 1999;22:119-124.
133. Raskin P, Jovanovic L, Berger S, Schwartz S, Woo V, Ratner R. Repaglinide/troglitazone combination therapy: improved glycemic control in type 2 diabetes. *Diabetes Care*. 2000;23:979-983.
134. Horton ES, Clinkingbeard C, Gatlin M, Foley J, Mallows S, Shen S. Nateglinide alone and in combination with metformin improves glycemic control by reducing mealtime glucose levels in type 2 diabetes. *Diabetes Care*. 2000;23:1660-1665.
135. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest*. 1999;104:787-794.
136. Kahn SE, Prigeon RL, Schwartz RS, et al. Obesity, body fat distribution, insulin sensitivity and Islet beta-cell function as explanations for metabolic diversity. *J Nutr*. 2001;131:354S-360S.
137. Pigon J, Giacca A, Ostenson CG, Lam L, Vranic M, Efendic S. Normal hepatic insulin sensitivity in lean, mild noninsulin-dependent diabetic patients. *J Clin Endocrinol Metab*. 1996;81:3702-3708.
138. Kuzuya T, Kosaka K, Akanuma Y, Shigeta Y, Kaneko T. Baseline factors affecting the efficacy of troglitazone on plasma glucose in Japanese patients with non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract*. 1998;41:121-129.
139. Turner RC, Cull CA, Frighi V, Holman RR, and the UKPDS Group. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus. *JAMA*. 1999;281:2005-2012.
140. Erle G, Lovise S, Stocchiero C, et al. A comparison of preconstituted, fixed combinations of low-dose glyburide plus metformin versus high-dose glyburide alone in the treatment of type 2 diabetic patients. *Acta Diabetol*. 1999;36:61-65.
141. Costa B, Pinol C. Acarbose in ambulatory treatment of non-insulin-dependent diabetes mellitus associated to imminent sulfonylurea failure: a randomized-multicentric trial in primary health-care: Diabetes and Acarbose Research Group. *Diabetes Res Clin Pract*. 1997;38:33-40.
142. Rosenstock J, Brown A, Fischer J, et al. Efficacy and safety of acarbose in metformin-treated patients with type 2 diabetes. *Diabetes Care*. 1998; 21:2050-2055.
143. Scorpiglione N, Belfiglio M, Carinci F, et al. The effectiveness, safety and epidemiology of the use of acarbose in the treatment of patients with type II diabetes mellitus: a model of medicine-based evidence. *Eur J Clin Pharmacol*. 1999;43:179-185.
144. Holman RR, Cull CA, Turner RC. A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years (UKPDS 44). *Diabetes Care*. 1999;22:960-964.
145. Willms B, Ruge D. Comparison of acarbose and metformin in patients with type 2 diabetes mellitus insufficiently controlled with diet and sulphonylureas: a randomized, placebo-controlled study. *Diabet Med*. 1999;16:755-761.
146. Standl E, Schernthaner G, Rybka J, Hanefeld M, Raptis SA, Naditch L. Improved glycaemic control with miglitol in inadequately-controlled type 2 diabetics. *Diabetes Res Clin Pract*. 2001;51:205-213.
147. Iwamoto Y, Kosaka K, Kuzuya T, Akanuma Y, Shigeta Y, Kaneko T. Effect of combination therapy of troglitazone and sulphonylureas in patients with type 2 diabetes who were poorly controlled by sulphylurea therapy alone. *Diabet Med*. 1996;13:365-370.
148. Buysschaert M, Bobbioni E, Starkie M, Frith L. Troglitazone in combination with sulphonylurea improves glycaemic control in type 2 diabetic patients inadequately controlled by sulphonylurea therapy alone: Troglitazone Study Group. *Diabet Med*. 1999; 16:147-153.
149. Bando Y, Ushiogi Y, Okafuji K, Toya D, Tanaka N, Fujisawa M. Troglitazone combination therapy in obese type 2 diabetic patients poorly controlled with alpha-glucosidase inhibitors. *J Int Med Res*. 1999;27: 53-64.
150. Einhorn D, Rendell M, Rosenzweig J, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride in combination with metformin in the treatment of type 2 diabetes mellitus: a randomized, placebo-controlled study: the Pioglitazone 027 Study Group. *Clin Ther*. 2000;22:1395-1409.
151. Wolffenbuttel BH, Gomis R, Squatrito S, Jones NP, Patwardhan RN. Addition of low-dose rosiglitazone to sulphonylurea therapy improves glycaemic control in type 2 diabetic patients. *Diabet Med*. 2000;17:40-47.
152. Yale JF, Valiquett TR, Ghazzi MN, Owens-Grillo JK, Whitcomb RW, Foyt HL. The effect of a thiazolidinedione drug, troglitazone, on glycemia in patients with type 2 diabetes mellitus poorly controlled with sulfonylurea and metformin: a multicenter, randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2001;134:737-745.
153. Berger M, Jorgens V, Muhlhauser I. Rationale for the use of insulin therapy alone as the pharmacological treatment of type 2 diabetes. *Diabetes Care*. 1999;22(suppl 3):C71-C75.
154. Buse J. Combining insulin and oral agents. *Am J Med*. 2000;108(suppl 6):23S-32S.

EXHIBIT D

Outpatient Insulin Therapy in Type 1 and Type 2 Diabetes Mellitus

Scientific Review

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Irl B. Hirsch, MD

Context Newer insulin therapies, including the concept of physiologic basal-prandial insulin and the availability of insulin analogues, are changing clinical diabetes care. The key to effective insulin therapy is an understanding of principles that, when implemented, can result in improved diabetes control.

Objective To systematically review the literature regarding insulin use in patients with type 1 and type 2 diabetes mellitus (DM).

Data Sources A MEDLINE search was performed to identify all English-language articles of randomized controlled trials involving insulin use in adults with type 1 or type 2 DM from January 1, 1980, to January 8, 2003. Bibliographies and experts were used to identify additional studies.

Study Selection and Data Extraction Studies were included (199 for type 1 DM and 144 for type 2 DM, and 38 from other sources) if they involved human insulins or insulin analogues, were at least 4 weeks long with at least 10 patients in each group, and glycemic control and hypoglycemia were reported. Studies of insulin-oral combination were similarly selected.

Data Synthesis Twenty-eight studies for type 1 DM, 18 for type 2 DM, and 48 for insulin-oral combination met the selection criteria. In patients with type 1 DM, physiologic replacement, with bedtime basal insulin and a mealtime rapid-acting insulin analogue, results in fewer episodes of hypoglycemia than conventional regimens. Rapid-acting insulin analogues are preferred over regular insulin in patients with type 1 DM since they improve HbA_{1c} and reduce episodes of hypoglycemia. In patients with type 2 DM, adding bedtime neutral protamine Hagedorn (isophane) insulin to oral therapy significantly improves glycemic control, especially when started early in the course of disease. Bedtime use of insulin glargine results in fewer episodes of nighttime hypoglycemia than neutral protamine Hagedorn regimens. For patients with more severe insulin deficiency, a physiologic insulin regimen should allow lower glycemic targets in the majority of patients. Adverse events associated with insulin therapy include hypoglycemia, weight gain, and worsening diabetic retinopathy if hemoglobin A_{1c} levels decrease rapidly.

Conclusions Many options for insulin therapy are now available. Physiologic insulin therapy with insulin analogues is now relatively simple to use and is associated with fewer episodes of hypoglycemia.

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PRIMARY CARE PHYSICIANS provide diabetes care for 39% of the 16 million patients in the United States (US) with type 1 diabetes mellitus (DM) and 82% of patients with type 2 DM.[1] The greatest change in diabetes therapy in the last decade has been the introduction of insulin analogues. Currently, 6 to 7 million Americans use human insulin or insulin analogues. The availability of the new insulin analogues makes physiologic insulin therapy realistic for many patients, because the onset and duration of the action of these analogues more closely mimic human insulin secretion, thus simplifying insulin dosing and adjustment and increasing flexibility for patients. The use of physiologic insulin replacement and continuous subcutaneous insulin infusion (CSII, or pump therapy) are increasingly popular and have become the criterion standard, with more than 200 000 patients with type 1 DM using CSII therapy worldwide.[2]

The American Diabetes Association recommends a hemoglobin (Hb) A_{1c} level less than 7%.[3] Data from 1988-1995[4] show that 43% of US patients had an HbA_{1c} level greater than 8.0%, 18% had poor control with an HbA_{1c} level greater than 9.5% (24% of the insulin-treated patients had poor control). More than 50% of US patients with type 1 DM

See also p 2265 and Patient Page.

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use only 1 to 2 insulin injections per day, a suboptimal, nonphysiologic approach to type 1 DM insulin therapy.[5] Importantly, even many patients with type 2 DM would not achieve adequate control using twice-daily neutral protamine Hagedorn (NPH or isophane insulin).[6]

Most physicians would agree that good diabetes control, which often requires intensive insulin therapy, is desirable for patients with type 1 DM and type 2 DM. Patients receiving intensive therapy with lower HbA_{1c} levels with type 1 DM in the Diabetes Control and Complications Trial, or with type 2 DM in the United Kingdom Prospective Diabetes Study (UKPDS), had fewer, later microvascular complications.[7,8] Interestingly, some data suggest that insulin may benefit patients with DM in other ways. For example, early insulin therapy may preserve β-cell function.[9,10] Insulin therapy can also improve lipid metabolism[11-15] and mortality after myocardial infarction.[16]

With diabetes-related medical costs of $132 billion per year (more than 12% of the US health care budget),[17] many experts question whether intensive insulin therapy (approximately $16 000-30 000 per quality-adjusted life years gained)[17] is cost-effective. In the UKPDS, the incremental yearly cost of intensive insulin therapy for patients with type 2 DM (either with sulfonylurea [SU] agents or with insulin) was $1866,[18] while in the Kumamoto trial, multiple injection therapy for patients with type 2 DM reduced costs from $31 525 for conventional therapy to $30 310, by decreasing complications.[19]

METHODS

We searched MEDLINE for all English-language articles involving insulin use in adults with type 1 DM (n=199) or type 2 DM (n=144) between January 1, 1980, and January 8, 2003. Bibliographies and experts allowed for the identification of additional relevant abstracts (n=3) and studies (n=35). Randomized controlled trials were included (28 for type 1 DM and 18 for type 2 DM) if they compared currently available human insulins, reported glucose measurements and/or rates of hypoglycemic episodes, and were at least 4 weeks long with at least 10 patients in each group. Using similar criteria, randomized controlled trials of insulin-oral agent combination therapy (n=48) were reviewed in detail. Studies with English-language abstracts or those using animal and human insulins were selected if they were included in previously published reviews or meta-analyses and met our other criteria.

The authors reviewed, summarized, and synthesized the data. We found the literature highly problematic because it lacked standardized medication protocols, methods, and end points. A large majority of trials were sponsored by the pharmaceutical industry. Given the paucity of evidence in some areas, we believe that expert clinical diabetes practice is far ahead of clinical trials.

Table 1. Currently Available Insulin Products*

Insulin†	Onset	Peak	Effective Duration, h	Cost per 10 mL per 100 U/mL‡
Rapid-acting	5-15 min	30-90 min	5	
Lispro (Humalog)				$46
Aspart (NovoLog)				$58
Short-acting	30-60 min	2-3 h	5-8	
Regular U100				$25
Regular U500 (concentrated)				$220/20 mL
Buffered regular (Velosulin)				$55
Intermediate-acting				
Isophane insulin (NPH, Humulin N/Novolin N)	2-4 h	4-10 h	10-16	$24-$26
Insulin zinc (Lente, Humulin L/Novolin L)	2-4 h	4-12 h	12-18	$24-$26
Long-acting				
Insulin zinc extended (Ultralente, Humulin U)	6-10 h	10-16 h	18-24	$25
Glargine (Lantus)	2-4 h§	No peak	20-24	$46
Premixed				
70% NPH/30% regular (Humulin 70/30)	30-60 min	Dual	10-16	$25
50% NPH/50% regular (Humulin 50/50)	30-60 min	Dual	10-16	$46
75% NPL/25% lispro (Humalog Mix 75/25)	5-15 min	Dual	10-16	$58
70% NP/30% aspart (NovoLog Mix)	5-15 min	Dual	10-16	$59

Abbreviations: L, Lente; NPH, neutral protamine Hagedorn; NPL, insulin lispro protamine (neutral protamine lispro).
*Adapted with permission from *Practical Insulin: A Handbook for Prescribing Providers*, The American Diabetes Association, 2002.[153]
†Assuming 0.1-0.2 U/kg per injection. Onset and duration vary significantly by injection site.
‡Prices are for comparison and may vary widely. Sources of prices are from Drugstore.com (http://www.drugstore.com) or retail ranges from Costco, Safeway, Rite Aid, and Walgreens.
§Time to steady state.

RESULTS

What Are the Major Types of Insulin?

Rapid-Acting Insulin. Insulin lispro and insulin aspart do not self-aggregate in solution as human (regular) insulin does, and these insulins are rapidly absorbed (TABLE 1). Insulin lispro differs from human insulin by an amino acid exchange of lysine and proline at positions 28 and 29. The substitution of aspartic acid for proline at position 28 created insulin aspart. Rapid-acting insulins are most appropriately injected at mealtime as "prandial" insulin (sometimes referred to as "bolus" insulin) or used in insulin pumps.

Short-Acting Insulin. Regular insulin has a delay to onset of action of 30 to 60 minutes (Table 1). Patients are instructed to inject regular insulin 20 to 30 minutes prior to meals (ie, lag time is the time between injecting insulin and eating) to match insulin availability and carbohydrate absorption. Regular insulin acts almost immediately when injected intravenously.

Intermediate-Acting Insulin. Neutral protamine Hagedorn (isophane insulin; NPH) insulin is slowly absorbed due to the addition of protamine to regular insulin (Table 1). Regular insulin bound to zinc, Lente insulin, has a slightly longer effective duration than

NPH. Lente and NPH are commonly used as twice-daily basal insulins. Neutral protamine lispro (insulin lispro protamine; NPL) and protamine crystalline (crystal) aspart, available in the United States only in premixed insulins, are functionally identical to NPH.

Long-Acting Insulin. Ultralente insulin (insulin zinc extended) is absorbed slowly in its zinc crystalline form. Insulin glargine, a modified human insulin that forms a microprecipitate in the subcutaneous tissue, is released slowly with a peakless delivery of about 20 to 24 hours in most patients (Table 1).

What Are the Major Adverse Effects of Insulin?

Hypoglycemia is the most common adverse effect of insulin therapy. In the Diabetes Control and Complications Trial (type 1 DM),[20] intensive therapy increased the risk of severe hypoglycemia, defined as needing the assistance of another person. Severe hypoglycemia was reported by 26% of patients with a mean of 1.9 episodes per patient per year, and 43% of episodes occurred nocturnally. In the UKPDS, patients with type 2 DM receiving insulin therapy had lower HbA_{1C} levels, but 1% to 2% more patients receiving insulin reported at least 1 episode of severe hypoglycemia per year than those patients receiving other therapies. Intensive therapy, with oral medications or insulin, has been shown to increase the risk of episodes of hypoglycemia.[8]

Generally, patients receiving insulin gain weight. As patients attempt better glycemic control, decreased glycosuria and intermittent overinsulinization can result in hypoglycemia, hunger, and increased caloric intake. In the Diabetes Control and Complications Trial, patients with type 1 DM receiving intensive insulin therapy gained +.75 kg more than patients receiving conventional therapy during the 3.5- to 9-year study period ($P<.001$), although waist-hip ratios did not differ between groups.[21] In the UKPDS, patients with type 2 DM receiving intensive insulin therapy gained significantly more weight (1.4-2.3 kg) than those patients treated with SUs or metformin.[8] Bedtime administration of NPH produces less weight gain than daytime NPH, making bedtime administration a preferred strategy when starting insulin therapy in patients with type 2 DM.[22,23] In one study, patients gained less weight with insulin glargine than with conventional therapy with NPH.[24]

Rapid improvement in diabetes control results in progressive worsening of retinopathy in approximately 5% of patients.[25-27] Patients with proliferative retinopathy and who have an HbA_{1C} level greater than 10% are at highest risk of worsening retinopathy.[28] In these patients, we recommend reducing the HbA_{1C} level slowly (2% each year) with frequent ophthalmologic examinations (eg, every 6 months or for any symptoms) to ensure aggressive treatment of progressive retinopathy.

What Are the Major Issues Regarding Insulin Delivery?

When prescribing insulin for patients, important issues include insulin pharmacokinetics and compatibility, technological issues, and costs. Insulin absorption variability is the biggest confounder of efforts to mimic physiologic insulin secretion. The onset and duration of action of types of insulin vary greatly when different insulins are mixed, by injection site, and among patients.[29] Large doses of human insulins form an insulin depot, unpredictably prolonging the duration of action; this response is less of an issue for the insulin analogues.[30] Thus, patients injecting 40 U of NPH insulin into their abdominal region before breakfast may have a significantly different onset and peak of action than the same patients injecting 20 U of NPH in their thigh in the evening; mixing insulin lispro with the morning NPH dose and regular with the evening NPH dose would result in further variation. Insulin glargine may not be mixed with other insulins. Cloudy insulins, for example NPH, must be resuspended before administration, and if done improperly the insulin concentration may vary significantly.[31] Importantly, any strategy that increases the consistency of delivery should decrease glucose fluctuations.

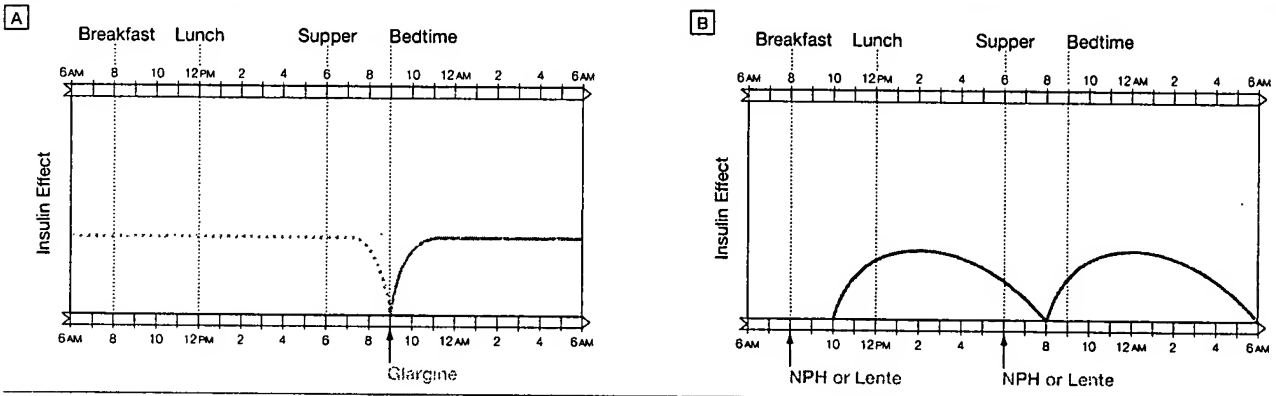
Insulin pens are convenient and may help avoid some insulin errors, but insulin cartridges for pens are more expensive than insulin in vials. Patients using insulin pumps must attend to tubing and injection site issues, must closely monitor their blood glucose level, and should have a back-up method of insulin administration.

What Are the Differences Between Physiologic and Nonphysiologic Insulin Regimens?

We refer to regimens that do not mimic normal β-cell secretion as "nonphysiologic insulin replacement" (FIGURE 1). "Physiologic insulin replacement" attempts to mimic normal insulin secretion. In general, physiologic regimens replace basal and prandial insulin (often referred to as "bolus") separately. In our experience, physicians and patients frequently misunderstand this key difference.

Traditionally, NPH was the primary basal insulin and regular was the primary prandial insulin. However, as typically used, each provides both basal and prandial effects. In conventional twice-daily NPH and regular insulin regimens (FIGURE 2), morning regular insulin is responsible for glucose disposal for breakfast, but its effective duration of 5 to 8 hours also makes it prandial insulin at lunch. After the absorption of breakfast (carbohydrate disposal is usually complete by midmorning), the regular insulin becomes, by definition, basal insulin. The morning NPH insulin is basal insulin after breakfast and lunch absorption are complete, and becomes the primary prandial insulin for lunch. But the relatively quick onset of NPH makes it functionally a component of the breakfast prandial insulin. This regimen requires strict consistency of the timing of injections and meals. Delaying lunch frequently results in hypoglycemia, at least for many patients trying to achieve meticulous glycemic control. Because NPH and regular insulin overlap in the later part

Figure 1. Examples of Nonphysiologic Insulin Replacement

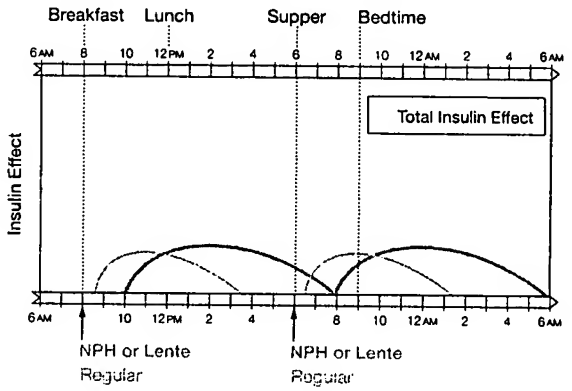


Nonphysiologic insulin replacement does not mimic normal β-cell insulin secretion. A, Once-daily, long-acting insulin glargine is released with a peakless delivery of approximately 20 to 24 hours in most patients. Glargine achieves steady state at approximately 2 hours. Dashed line indicates the effective duration of glargine continuing through the following day. B, Twice-daily, intermediate-acting neutral protamine Hagedorn (isophane insulin; NPH) and Lente (insulin zinc) are commonly used as basal insulin. Arrows indicate insulin injection.

of the morning, many patients require midmorning snacks to prevent hypoglycemia (Figure 2).

Using prandial insulin for each meal (either regular insulin, insulin lispro, or insulin aspart) with separate basal insulin (NPH, Lente, Ultralente, or insulin glargine) adds flexibility to the regimen, and glargine-lispro or glargine-aspart regimens allow patients to skip meals or change mealtimes (FIGURE 3). This approach requires more injections than with conventional twice-daily physiologic regimens, but surveys show that patients with type 1 DM are injecting insulin more frequently and they prefer the dietary freedom, with education about more complex strategies for their care, rather than simplistic rules.[1,32] In one study, 80% of patients preferred a qualitative strategy and 20% preferred a quantitative strategy to a "simple" but relatively inflexible strategy.[33] Dose adjustment is much simpler with true basal-prandial regimens (eg, glargine-lispro) than with insulins that function as both a basal and a prandial insulin (eg, NPH).

Figure 2. Example of Conventional Physiologic Insulin Regimen



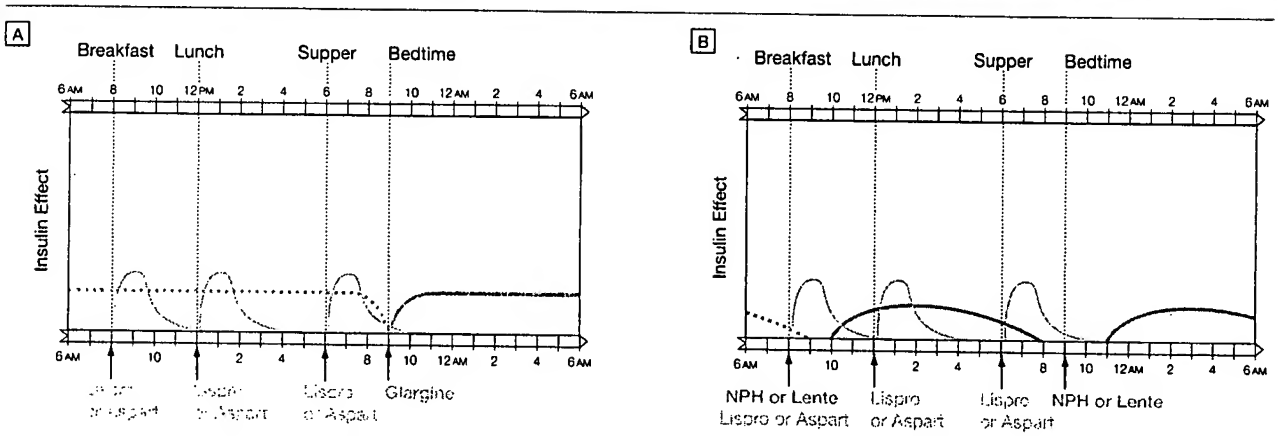
Physiologic insulin replacement with intermediate-acting neutral protamine Hagedorn (isophane insulin; NPH) or Lente (insulin zinc) and short-acting regular insulin (shown in a ratio of 70:30) attempts to mimic normal β-cell insulin secretion. Each insulin serves as both a basal and a prandial insulin. Meal timing and consistency are important for patients using this regimen. Many patients require a midmorning and bedtime snack to prevent hypoglycemia when the effect of the 2 insulins overlap at late morning and nighttime. Moving the dinnertime NPH injection to bedtime decreases the risk of nocturnal hypoglycemia. Arrows indicate insulin injection.

How Does the Patient Use Supplements and Adjustments?

Hyperglycemia correction is an important principle of insulin therapy. A supplement is a predetermined dose of rapid- or short-acting insulin used to correct hyperglycemia. Supplements are easier to determine when basal and prandial insulins are administered separately. Supplements are usually injected with the usual prandial dose of insulin. A conservative dose for patients with type 1 DM is an additional 1 U per 50 mg/dL (2.7 mmol/L) above the target blood glucose level. For patients with type 2 DM, we recommend 1 U of supplemental insulin per 30 mg/dL (1.7 mmol/L) above the target glucose level.

If patients are using insulin supplements between meals, they must beware of "insulin stacking." Injecting additional short- or rapid-acting insulin 1 hour after a dose of regular and NPH insulin would result in insulin stacking and in predictable hypoglycemia within several hours because most of the previously injected insulin has not been ab-

Figure 3. Examples of Physiologic Insulin Delivery Regimen



A, Once-daily glargine with lispro or aspart (shown in a ratio of 50:50) allows patients to skip meals or change mealtimes. Insulins lispro and aspart (rapid acting) are prandial insulins and glargine (long acting) is a basal insulin. This regimen is easier to use since it has true basal and prandial insulins. Dashed line indicates the effective duration of glargine continuing through the following day. Glargine achieves steady state at approximately 2 hours. B, Intermediate-acting neutral protamine Hagedorn (isophane insulin; NPH) and Lente (insulin zinc) are basal insulins. Rapid-acting lispro and aspart insulins are prandial insulins. This regimen (shown in a ratio of 50:50) is more difficult to adjust because NPH can act as both a basal and a prandial insulin. Dashed line indicates the effective duration of NPH or Lente continuing through the following day. Arrows indicate insulin injection.

sorbed. If patients are to inject supplements less than 3 hours after a previous insulin dose, they can decrease the supplement by 50%. Patients who exercise may be required to adjust their dose of rapid-acting insulin analogues. Patients who exercise early in the postprandial period (1-3 hours) may need to decrease their dose of rapid-acting insulin by 75%, whereas patients who exercise later in the postprandial period may require a smaller or no change in dose.[34,35]

An "adjustment" means changing the dose of any type of insulin based on a consistent pattern of blood glucose levels. For example, the adjustment for a patient receiving bedtime NPH insulin who has frequent fasting hypoglycemia would be to decrease the bedtime insulin dose. Aggressive but careful adjustments based on patients' injection timing meal patterns and activity levels are key to excellent long-term glucose control.

Why Is It Important for Patients to Self-monitor?

While there is little controversy that all patients receiving insulin should perform self-monitoring of blood glucose tests, there is disagreement about the frequency and timing of the tests. For type 1 DM, the American Diabetes Association suggests 3 or more tests per day.[29] The data are less clear for patients with insulin-requiring type 2 DM. Many type 2 DM studies exclude patients receiving insulin, lump insulin users and nonusers, and were conducted before the availability of insulin analogues and improved self-monitoring of blood glucose equipment. A recent study suggests self-monitoring of blood glucose is associated with improved control in patients with type 2 DM who use the results to adjust insulin doses.[36]

What Regimens Are Best for Patients With Type 1 DM?

Type 1, autoimmune, DM occurs in adults of all ages, including obese patients with phenotypic type 2 DM. Latent autoimmune DM (also known as LADA) of adults can be confused with type 2 DM early in diagnosis, but patients become insulinopenic relatively rapidly.[37]

Nonphysiologic Regimens. Some newly diagnosed patients with type 1 DM or latent autoimmune DM of adults who are still producing endogenous insulin may do well receiving once- or twice-daily basal insulin injections before they progress to complete β-cell failure (Figure 1). The time to complete insulin deficiency varies, but it is generally longer in adults than in children. Even with euglycemia, few physicians would recommend discontinuing insulin completely because intensive insulin therapy appears to promote β-cell preservation.[9,10,38] Data are not available to date to compare different nonphysiologic insulin regimens in this patient population.

Physiologic Regimens (TABLE 2). In patients with severe insulin deficiency, replacement of both prandial and basal insulin components is required. In patients with type 1 DM and no endogenous insulin secretion, it is very difficult to safely reach target HbA_{1c} level (<7%) with conventional insulin therapy, twice-daily NPH, and regular insulin (as shown in Figure 2). This regimen is difficult to adjust, and it is relatively inflexible because it uses both insulin components as both a prandial and a basal insulin. Moving NPH insulin from dinnertime to bedtime was first suggested in the 1980s as a strategy to optimize this conventional regimen.[39] Mixed NPH and regular insulin are given before breakfast, regular insulin is injected before dinner, and NPH is given at bedtime. A recent randomized, crossover study confirmed that this bedtime

Table 2. Available Insulin Delivery Systems and the Cost of a Physiologic Regimen With Each System

Delivery System	Advantages	Disadvantages	Cost (Comparative Examples for Initial and Monthly Cost)* Item	Amount
Syringe	Maximal ability to "freemix" insulin and adjust to patient needs	Multiple injections Need to carry bottles, syringes, and supplies Variable absorption depending on type of insulin and body injection site Lispro and glargine are both clear insulins and therefore difficult to distinguish, patients must read labels carefully	Insulin glargine 1000 U	$44
			Insulin lispro 1000 U	$46
			Syringes for 4 injections/d (120-gauge)	$36
			Total cost per month for glargine at bedtime + lispro 3 times/d	$126 Bedtime NPH + 3 times/d of prandial regular = $25 + $25 + $36 = $86
Pen	Convenient, less to carry Easy to distinguish between insulins by pen color/size Improves dosing accuracy Although not recommended, many use 1 needle per 24 h	For injection, approximately 30% more expensive per 1000 U than bottled insulin	Pen injector	Novopen 3 = $29-$32 retail
			Pen cartridges for 1000 U	NPH = $42 Glargine = $63 Lispro = $63
			Total cost per month for bedtime dose with needles	NPH/prandial lispro = $105 Glargine/lispro = $126
Pump	Fewer injections Physiologic delivery with best glycemic control and fewest hypoglycemic events overall Eliminates variable injection-site absorption	Expensive Additional training needed Patient must be aware of potential technical problems	Pump: Medtronic MiniMed	$5500/60 mo at $92 per month (assumes pump life of 5 years)
			Monthly cost of tubing/reservoirs	$150
			Insulin lispro 2000 U	$92
			Total cost per month	$334

Abbreviation: NPH, neutral protamine Hagedorn (isophane insulin).
*We estimated costs based on 0.9 U/kg for a 70-kg person at 63 U/d, 32 U of each per day, equals 1000 U/mo of each insulin type. Patients are told to discard their unused bottles at the end of the month if they use less insulin.

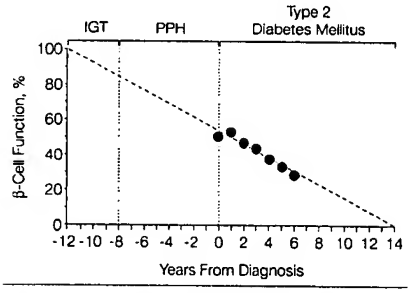
NPH strategy reduces both HbA_{1C} levels and nocturnal hypoglycemic episodes in patients with type 1 DM.[40]

Overall, patients using insulin analogues (lispro, aspart, glargine) in physiologic regimens (Figure 3A), including patients with hypoglycemia unawareness, have fewer hypoglycemic episodes than patients using traditional insulins (regular and NPH).[41-46] Because of shorter duration of action, insulin lispro (introduced in the United States in 1996) and insulin aspart are only used as prandial insulins or in CSII programs. When patients use insulins lispro or aspart, they have fewer episodes of severe hypoglycemia and nocturnal hypoglycemia than with regular insulin[47-50] (eTABLE 1[42-44,46,50-72] and eTABLE 2[12,24,27,41,53-67,72-83]; tables are published online at http://www.jama.com). Lag time depends on the onset of action of the prandial insulin used (eg, 30 minutes for regular insulin and none for insulin lispro or aspart). An inadequate lag time results in postprandial hyperglycemia and in later risk of hypoglycemia. Patient compliance with the recommended 30-minute lag time for regular insulin is 30% to 70% (patients inject insulin closer to or at mealtime.[84,85] The lack of required lag time for rapid-acting insulins and improved matching of action with carbohydrate absorption explain their clinical advantage (Figure 3).

Data on regimens using rapid-acting analogues with basal NPH are mixed (Figure 3B). Improvements in HbA_{1C} levels have not been seen when analogues are given with basal NPH provided once or twice daily, because the improvement in postprandial hyperglycemia seen with the rapid-acting analogues is negated by higher preprandial and overnight glycemia (compared with regular insulin). One study using small doses of NPH given with insulin lispro before each meal and at bedtime, to better control basal needs between meals, showed decreased HbA_{1C} levels and episodes of hypoglycemia.[62] However, in a recent study of patients with type 1 DM receiving NPH basal insulin (1-2 injections per day) with prandial lispro, adding an additional injection of NPH at lunchtime in an attempt to give smoother basal control resulted in 6.9 more episodes of severe hypoglycemia per patient-year ($P = .007$).[86] Ultralente, which is longer acting than NPH or Lente, was developed to improve basal insulin delivery. However, twice-daily Ultralente, as compared with Lente, mildly improves fasting glucose levels but increases episodes of hypoglycemia.[87]

Insulin glargine became available in the United States in 2001. Theoretically, this peakless, long-acting basal insulin analogue should reduce hypoglycemia and improve glycemic control.[88] In actuality, reductions in episodes of hypoglycemia, especially nocturnal hypoglycemia, occur consistently whereas reductions in HbA_{1C} levels have been more difficult to achieve (eTables 1 and 2; tables are published online at http://www.jama.com). A large multicenter trial of patients with type 1 DM using insulin glargine with prandial regular insulin showed no change in HbA_{1C} levels, although 25% fewer hypoglycemic episodes were noted.[42] When insulin lispro was used as the prandial insulin, no differences in HbA_{1C} levels or hypoglycemic epsisodes were observed, but patients receiving glargine gained slightly less weight.[63] When glargine and lispro were compared with NPH and regular insulin in adoles-

Figure 4. Progressive Decline in β-Cell Function and Insulin Secretion in Type 2 Diabetes Mellitus



Data show 50% of normal β-cell function at diagnosis of type 2 diabetes mellitus (year 0) and a steady decline up to 6 years following diagnosis. Clinically, most patients have had prediabetes (impaired glucose tolerance [IGT] and postprandial hyperglycemia [PPH]) for some time before clinical diagnosis of type 2 diabetes mellitus. Dotted line shows the extrapolation of β-cell function before and after diagnosis of diabetes. Adapted with permission from *Diabetes Reviews*[133] based on data from the United Kingdom Prospective Diabetes Study.[92]

cents, results of HbA_{1c} levels were similar, but the glargine-lispro regimen produced fewer hypoglycemic episodes.[61] However, in a population with a lower baseline HbA_{1c} level (7.1%), substituting insulin glargine for NPH, with prandial insulin lispro, decreased hypoglycemic episodes and HbA_{1c} levels.[89]

It may be that the main impact of physiologic insulin regimens and insulin glargine in particular is that the separation of prandial and basal components improves our understanding of insulin use, simplifies dosing adjustments, and allows patients more flexibility in meal timing. With a distinctly different basal insulin component (glargine or pump therapy), patients need approximately half of their insulin as basal insulin. When initiating a basal-prandial regimen, patients should decrease the calculated 50% basal insulin dose by 20% to avoid hypoglycemia. Using this calculation, one third of patients are receiving the correct dose, one third need more, and one third need less basal insulin.[90]

When Should Insulin Be Used in Type 2 DM?

Most patients with type 2 DM will eventually need insulin. Insulin therapy was started in patients with type 2 DM with a mean HbA_{1c} level of 10.4% in the United States,[91] and the UKPDS[92] showed that β-cell failure is progressive; 50% of normal β-cell function at diagnosis with a steady decline following diagnosis (FIGURE 4). Concomitantly, 53% of patients with type 2 DM initially treated with SUs required insulin therapy by 6 years, and almost 80% required insulin by 9 years.[93,94] Although we may be diagnosing DM earlier and thus altering this time frame, physicians should consider starting insulin therapy in patients whose HbA_{1c} level approaches 8% despite optimal oral therapy.

Improved glycemic control delays or prevents complications in patients with type 2 DM,[8,95,96] although patients often need an insulin dosage of greater than 100 U/d to achieve glycemic control.[94] Patients with type 2 DM often resist physician recommendations to start insulin therapy, partly because of misperceptions that starting insulin means the patient and physician have failed. Several unmasked studies suggest that switching from oral agents to the use of insulin in patients with type 2 DM improves treatment satisfaction, general well being, and quality of life, especially if patients previously had poor glycemic control.[22,75,79,97] When choosing an insulin regimen, the benefits of intensive therapy must be tempered by cost and ease of regimen. In general, treatment satisfaction is better with simpler regimens. Patients allocated to strict control (fasting plasma glucose level <117 mg/dL [6.5 mmol/L]) or less strict control (fasting plasma glucose level <153 mg/dL [8.5 mmol/L]) for 1 year reported improved mood and general well being if their HbA_{1c} level decreased 1% or more, but strict targets increased perceived treatment burden.[98] It has been shown that patients prefer insulin glargine to NPH,[66] twice-daily NPH to Ultralente, and insulin pen administration or premixed insulin to free-mixed insulin administered with syringes.[99-103]

What Is the Best Regimen for Patients With Type 2 DM?

Combination Oral Agent/Insulin Therapy. When using bedtime basal insulin (NPH or glargine), continuing 1 or 2 daytime oral medications is reasonable (eTABLE 3[6,8,15,94,97,104-132,134-146]; table is published online at http://www.jama.com). Metformin with insulin results in similar metabolic control, less weight gain, lower insulin doses, and fewer hypoglycemic episodes than insulin alone or insulin/SU therapy.* Thus, metformin and insulin may be the best combination for the majority of patients with type 2 DM who do not have contraindications. However, it should be emphasized that the goal is the target HbA_{1c} level, not lower insulin dose. Patients who must discontinue metformin because of increasing plasma creatinine levels should have their insulin dose increased 20% to 36% to maintain glycemic control.[147]

Combining SUs with insulin lowers insulin doses (25%-50%) with less weight gain, but increases cost.† Sulfonylureas increase endogenous insulin secretion (C-peptide) early in the disease process. Improvement of HbA_{1c} with SU use in the UKPDS was in patients whose HbA_{1c} levels were well below 10%.[93] As insulin production declines and HbA_{1c} levels approach 10%, the combination of insulin and SUs eventually becomes ineffective.[149]

Insulin secretagogues include the SUs and the glinides. Glinides are functionally short-acting SUs and may improve prandial control with or without basal insulin. Not enough data are available to date to endorse their use,[150] especially given their cost, although they may be beneficial in patients with hypoglycemia or who skip meals.

Although thiazolidinediones (TZDs) are effective insulin sensitizers, combined TZD/insulin therapy has been problematic, and TZDs are expensive. Troglitazone was taken off the market due to liver failure, but one randomized trial comparing intensive insulin monotherapy vs insulin with either metformin or troglitazone showed that all therapies lowered HbA_{1c} levels effectively.[151] Patients gained about 4.4 kg while receiv-

*References 97, 113, 114, 119, 120, 140, 144.
†References 105, 110, 119, 123-125, 127, 148.

ing insulin or insulin/troglitazone, but only 0.5 kg while receiving insulin/metformin. Troglitazone significantly reduced the dose of insulin but caused the same rate of hypoglycemic episodes as insulin (2 per month), while patients receiving insulin/metformin reported no hypoglycemia. Pioglitazone and rosiglitazone should not be used with patients in New York Heart Association (NYHA) class III or IV heart failure, and patients' liver function must be monitored. Significant weight gain, pulmonary edema, and heart failure are increasingly associated with TZDs.[152] Given these issues, combination TZD/insulin therapy should be used with caution.

Insulin Therapy. The goals of insulin therapy in both type 1 and type 2 DM are to reach the target HbA_{1C} level with a low rate of hypoglycemic episodes and the least amount of weight gain (eTable 2; table is published online at http://www.jama.com). However, goals must be individualized since older patients with type 2 DM and with no complications may not benefit from intensive therapy. When starting insulin therapy in patients continuing daytime insulin secretagogues or metformin, with an HbA_{1C} level less than 9.5% to 10%, bedtime basal insulin therapy is effective, convenient, and produces less weight gain.[22,23,73] Compared with NPH, basal insulin glargine is associated with 25% fewer nocturnal hypoglycemic episodes, better postdinner control, and slightly less weight gain at twice the cost.[24,41] Both NPH and glargine are easily adjusted based on fasting blood glucose levels. Once-daily Ultralente insulin produces more hypoglycemic episodes than twice-daily NPH despite a higher HbA_{1C} level.[75] If nocturnal hypoglycemia is an issue and glargine is not an option, prandial lispro with SU lowers HbA_{1C} levels with fewer hypoglycemic episodes than NPH with SU.[146]

With progressive β-cell exhaustion, patients will be more successful in achieving glycemic control with progressively more physiologic regimens. Premixed insulins, given twice daily, (70% NPH/30% regular [70N/30R], 70% NP [neutral protamine]/30% aspart [A] [BIAsp], and 75% NPL/25% lispro [L]) are convenient but no prandial insulin is given for lunchtime. BIAsp improves postbreakfast/dinner blood glucose levels, but not HbA_{1C} levels, and decreases severe hypoglycemic episodes by 50% when compared with 70N/30R. Patients who are uncontrolled (ie, not achieving glycemic control) receiving premixed insulin regimens can often achieve control at the same insulin dose by adding lunchtime prandial insulin and by decreasing the morning insulin accordingly. Prandial insulin lispro is associated with fewer episodes of nocturnal hypoglycemia than regular insulin.[82] Another trial of lispro vs regular, with twice-daily basal Lente or Ultralente, showed a lower HbA_{1C} level with lispro at similar insulin doses.[67] Prandial therapy with lispro vs bedtime therapy with NPH lowers HbA_{1C} levels without additional hyperglycemia.[81] Importantly, patients with type 2 DM may require large insulin doses (>1 U/kg) to reach an HbA_{1C} level less than 7%.[6,153,154]

What Are the Advantages of Insulin Pump Therapy?

Patients with type 1 DM receiving CSII therapy show more improvement in HbA_{1C} levels than patients receiving intensive multiple injection therapy[155]; but it remains to be seen whether CSII will reduce the risk of microvascular complications. Compared with multiple injection therapy, CSII reduces hypoglycemic events up to 74%.[155] Intensive insulin therapy reduces costs by decreasing complications; and a study of CSII vs multiple injection therapy in peripartum patients with type 1 DM shows equal costs, but patients preferred pump therapy.[156]

An external pump is programmed to deliver individualized basal rates of short- or rapid-acting insulin (usually 0.5-1.5 U/h). Since patients receiving CSII need less insulin, it has been recommended to decrease the total daily dose by 20% to 30% and then use 50% of that reduced dose as basal insulin.[2] Prandial (bolus) insulin is given by manual activation. Rapid-acting insulins have been shown to be superior to regular insulin in a CSII program because of improved prandial control.[52,70]

The main indications for pump use in patients with type 2 DM without significant C-peptide secretion are severe hypoglycemia and wide fluctuations of glucose levels.[27,80] However, physiologic regimens with insulin glargine and lispro or aspart probably offer the same benefits at lower cost, albeit with more injections.

What Other Approaches Improve Outcomes or Reduce Costs?

While the practice of diabetes care is now increasingly precise, the complexities of care and compliance issues are overwhelming for many physicians. Improving systems of diabetes care may improve glycemic control compared with standard care as shown by (1) frequent insulin dose adjustment by nurse educators via telephone lowered the HbA_{1C} level from 9.4% to 7.8% (0.3% more than standard care)[157]; (2) "telecare" (transmitted data and telephone advice) improved HbA_{1C} levels 1% (vs 1.2%) and saved patients considerable travel time[158]; and (3) using computer decision models for adjustments of insulin doses lowered HbA_{1C} levels approximately 12% and decreased the rate of hypoglycemic episodes by 50% per week.[159,160]

COMMENT

An HbA_{1C} level less than 7% consistently reduces microvascular complications and is now the goal for most patients. Limited data suggest that reducing complications also reduces costs. A team approach with diabetes educators may be more effective at reducing complications at a similar cost. The lack of resources for efficient team care is a major barrier to diabetes care, especially in the primary care community.

Patients with type 1 DM almost always require multiple injections to attain an HbA_{1C} level less than 7%. Physiologic basal-prandial regimens are easier to use and adjust and cause fewer episodes of hypoglycemia. They also provide patients with more flexibility, and

studies on patient satisfaction support their use. However, insulin analogues cost 50% more than human insulins.

Patients with type 2 DM who still secrete endogenous insulin often do well receiving oral agents. The choice of oral agent depends largely on adverse effects and cost.[161] Oral agents alone lower HbA_{1c} levels 1% to 2%. Adding bedtime insulin, usually NPH, to oral agents is the standard approach to starting insulin therapy. Although insulin may be added to any approved oral agent, metformin does not cause weight gain and may offer additional cardioprotection[162] and thus is our first choice for use with insulin in patients without contraindications. Oral agents lower the required insulin dose. When patients with type 2 DM become insulin deficient, the principles of insulin use are the same as for patients with type 1 DM. Importantly, patients with type 2 DM often require large insulin doses, for example, 1 to 2 U/kg per day, and the use of lower doses in clinical practice is a common barrier to effective diabetes control.

Finally, treatment goals and intensity of insulin therapy must be individualized since young patients may benefit the most from intensive therapy and even expensive therapies may be cost-effective, while older patients without complications may not benefit as much from intensive therapy with its attendant risks.

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REFERENCES

1. Roper ASW. *U. S. Diabetes Patient Market Study.* By permission of East Hanover, NJ: Market Measures/ Cozint, an NOP World Co; 2002.
2. Bode BW, Sabbah HT, Gross TM, Fredrickson LP, Davidson PC. Diabetes management in the new millennium using insulin pump therapy. *Diabetes Metab Res Rev* 2002;18(suppl 1):S14-S20.
3. American Diabetes Association. Clinical practice recommendations 2002. *Diabetes Care.* 2002;25(suppl 1):S1-S147.
4. Saaddine JB, Engelgau MM, Beckless GL, Gregg EW, Thompson TJ, Narayan KM. A diabetes report card for the United States: quality of care in the 1990s. *Ann Intern Med.* 2002;136:565-574.
5. Tabak AG, Tamas G, Zgibor J, et al. Targets and reality. *Diabetes Care.* 2000;23:1284-1289.
6. Abraira C, Henderson WG, Colwell JA, et al. Response to intensive therapy steps and to glipizide dose in combination with insulin in type 2 diabetes. *Diabetes Care.* 1998;21:574-579.
7. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. *Ann Intern Med.* 1998;128: 517-523.
8. Intensive blood-glucose control with sulfonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet.* 1998;352:837-853.
9. Juneja R, Hirsch IB, Naik RG, et al. Islet cell antibodies and glutamic acid decarboxylase antibodies, but not the clinical phenotype, help to identify type 1(1/2) diabetes in patients presenting with type 2 diabetes. *Metabolism.* 2001;50:1008-1013.
10. Linn T, Ortac K, Laube H, Federlin K. Intensive therapy in adult insulin-dependent diabetes mellitus is associated with improved insulin sensitivity and reserve. *Metabolism.* 1996;45:1508-1513.
11. Rodier M, Colette C, Gouzes C, Michel F, Crastes de Paulet A, Monnier L. Effects of insulin therapy upon plasma lipid fatty acids and platelet aggregation in NIDDM with secondary failure to oral antidiabetic agents. *Diabetes Res Clin Pract.* 1995;28:19-28.
12. Landstedt-Hallin L, Adamson U, Arner P, Bolinder J, Lins PE. Comparison of bedtime NPH or preprandial regular insulin combined with glibenclamide in secondary sulfonylurea failure. *Diabetes Care.* 1995;18: 1183-1186.
13. Rivellese AA, Patti L, Romano E, et al. Effect of insulin and sulfonylurea therapy, at the same level of blood glucose control, on low density lipoprotein subfractions in type 2 diabetic patients. *J Clin Endocrinol Metab.* 2000;85:4188-4192.
14. Romano G, Patti L, Innelli F, et al. Insulin and sulfonylurea therapy in NIDDM patients. *Diabetes.* 1997; 46:1601-1606.
15. Wolffenbuttel BH, Weber RF, van Koetsveld PM, Weeks L, Verschoor L. A randomized crossover study of sulfonylurea and insulin treatment in patients with type 2 diabetes poorly controlled on dietary therapy. *Diabet Med.* 1989;6:520-525.
16. Malmberg K. Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. *BMJ.* 1997;314:1512-1515.
17. American Diabetes Association. Economic costs of diabetes in the US in 2002. *Diabetes Care.* 2003; 26:917-932.
18. Gray A, Raikou M, McGuire A, et al. Cost effectiveness of an intensive blood glucose control policy in patients with type 2 diabetes. *BMJ.* 2000;320:1373-1378.
19. Wake N, Hisashige A, Katayama T, et al. Cost-effectiveness of intensive insulin therapy for type 2 diabetes. *Diabetes Res Clin Pract.* 2000;48:201-210.
20. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. *Am J Med.* 1991; 90:450-459.
21. Influence of intensive diabetes treatment on body weight and composition of adults with type 1 diabetes in the Diabetes Control and Complications Trial. *Diabetes Care.* 2001;24:1711-1721.
22. Yki-Jarvinen H, Kauppala M, Kujansuu E, et al. Comparison of insulin regimens in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med.* 1992;327:1426-1433.
23. Yki-Jarvinen H. Comparison of insulin regimens for patients with type 2 diabetes. *Curr Opin Endocrinol Diabetes.* 2000;7:175-183.
24. Rosenstock J, Schwartz SL, Clark CM Jr, Park GD, Donley DW, Edwards MB. Basal insulin therapy in type 2 diabetes. *Diabetes Care.* 2001;24:631-636.
25. Helve E, Laati Kainen L, Merenmies L, Koivisto VA. Continuous insulin infusion therapy and retinopathy in patients with type I diabetes. *Acta Endocrinol (Copenh).* 1987;115:313-319.
26. Dahl-Jorgensen K, Brinchmann-Hansen O, Hanssen KF, Sarvik L, Aagenaes O. Rapid tightening of blood glucose control leads to transient deterioration of retinopathy in insulin dependent diabetes mellitus. *BMJ (Clin Res Ed).* 1985;290:811-815.
27. Jennings AM, Lewis KS, Murdoch S, Talbot JF, Bradley C, Ward JD. Randomized trial comparing continuous subcutaneous insulin infusion and conventional insulin therapy in type II diabetic patients poorly controlled with sulfonylureas. *Diabetes Care.* 1991; 14:738-744.
28. Chantelau E, Kohner EM. Why some cases of retinopathy worsen when diabetic control improves. *BMJ.* 1997;315:1105-1106.
29. Insulin administration. *Diabetes Care.* 2000;(23 suppl 1):S86-S89.
30. Galloway JA, Rost MA, Rathmacher RP, Carmichael RH. A comparison of acid regular and neutral regular insulin. *Diabetes.* 1973;22:471-479.
31. Jehle PM, Micheler C, Jehle DR, Breitig D, Boehm BO. Inadequate suspension of neutral protamine Hagedorn (NPH) insulin in pens. *Lancet.* 1999;354:1604-1607.
32. Training in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes. *BMJ.* 2002;325:746.
33. Kalergis M, Pacuad D, Strycher I, Meltzer S, Jones PJ, Yale JF. Optimizing insulin delivery. *Diabetes Obes Metab.* 2000;2:299-305.
34. Rabasa-Lhoret R, Bourque J, Ducros F, Chiasson JL. Guidelines for premeal insulin dose reduction for postprandial exercise of different intensities and durations in type 1 diabetic subjects treated intensively with a basal-bolus insulin regimen (ultralente-lispro). *Diabetes Care.* 2001;24:625-630.
35. Tuominen JA, Karonen SL, Malamies L, Bolli G, Koivisto VA. Exercise-induced hypoglycaemia in IDDM patients treated with a short-acting insulin analogue. *Diabetologia.* 1995;38:106-111.
36. Franciosi M, Pellegrini F, De Berardis G, et al. The impact of blood glucose self-monitoring on metabolic control and quality of life in type 2 diabetic patients. *Diabetes Care.* 2001;24:1870-1877.
37. Palmer JP, Hirsch IB. What's in a name. *Diabetes Care.* 2003;26:536-538.
38. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med.* 1993;329:977-986.
39. Francis AJ, Home PD, Hanning I, Alberti KG, Tunbridge WM. Intermediate acting insulin given at bedtime. *BMJ (Clin Res Ed).* 1983;286:1173-1176.
40. Fanelli CG, Pampenelli S, Porcellati F, Rossetti P, Brunetti P, Bolli GB. Administration of neutral protamine Hagedorn insulin at bedtime versus with dinner in type 1 diabetes mellitus to avoid nocturnal hypoglycemia and improve control. *Ann Intern Med.* 2002;136:504-514.
41. Yki-Jarvinen HA, Dressler, Ziemen M. Less nocturnal hypoglycemia and better post-dinner glucose control with bedtime insulin glargine compared with bedtime NPH insulin during insulin combination therapy in type 2 diabetes. *Diabetes Care.* 2000;23:1130-1136.
42. Ratner RE, Hirsch IB, Neifing JL, Garg SK, Mecca TE, Wilson CA. Less hypoglycemia with insulin glargine in intensive insulin therapy for type 1 diabetes. *Diabetes Care.* 2000;23:639-643.
43. Raskin P, Holcombe JH, Tamborlane WV, et al. A comparison of insulin lispro and buffered regular human insulin administered via continuous subcutane-

ous insulin infusion pump. *J Diabetes Complications*. 2001;15:295-300.
44. Pfutzner A, Kustner E, Forst T, et al. Intensive insulin therapy with insulin lispro in patients with type 1 diabetes reduces the frequency of hypoglycemic episodes. *Exp Clin Endocrinol Diabetes*. 1996;104:25-30.
45. Riddle MC, Rosenstock J, and H.S. Group. Treatment to target study. *Diabetes*. 2002;51(suppl 2): A113.
46. Ferguson SC, Strachan MW, James JM, Frier BM. Severe hypoglycaemia in patients with type 1 diabetes and impaired awareness of hypoglycaemia. *Diabetes Metab Res Rev*. 2001;17:285-291.
47. Brunelle BL, Llewelyn J, Anderson JH Jr, Gale EA, Koivisto VA. Meta-analysis of the effect of insulin lispro on severe hypoglycemia in patients with type 1 diabetes. *Diabetes Care*. 1998;21:1726-1731.
48. Heller SR, Amiel SA, Mansell P. Effect of the fast-acting insulin analog lispro on the risk of nocturnal hypoglycemia during intensified insulin therapy. *Diabetes Care*. 1999;22:1607-1611.
49. Holleman F, Schmitt HM, Rottier SR, Rees A, Symanowski S, Anderson JH. Reduced frequency of severe hypoglycemia and coma in well-controlled IDDM patients treated with insulin lispro. *Diabetes Care*. 1997; 20:1827-1832.
50. Home PD, Lindholm A, Riis A. Insulin aspart vs human insulin in the management of long-term blood glucose control in type 1 diabetes mellitus. *Diabet Med*. 2000;17:762-770.
51. Daniels AR, Bruce R, McGregor L. Lispro insulin as premeal therapy in type 1 diabetes. *N Z Med J*. 1997; 110:435-438.
52. Zinman B, et al. Insulin lispro in CSII: results of a double-blind crossover study. *Diabetes*. 1997;46:440-443.
53. Vignati L, Anderson JH Jr, Iversen PW. Efficacy of insulin lispro in combination with NPH human insulin twice per day in patients with insulin-dependent or non-insulin-dependent diabetes mellitus. *Clin Ther*. 1997;19:1408-1421.
54. Del Sindaco P, Ciofetta M, Lalli C, et al. Use of the short-acting insulin analogue lispro in intensive treatment of type 1 diabetes mellitus. *Diabet Med*. 1998; 15:592-600.
55. Home PD, Lindholm A, Hylleberg B, Round P. Improved glycemic control with insulin aspart. *Diabetes Care*. 1998;21:1904-1909.
56. Melki V, Ranard E, Lassmann-Vague V, et al. Improvement of HbA_{1c} and blood glucose stability in IDDM patients treated with lispro insulin analog in external pumps. *Diabetes Care*. 1998;21:977-982.
57. Colombel A, Murat J, Krompf M, Kuchly-Anton B, Charbonnel B. Improvement of blood glucose control in type 1 diabetic patients treated with lispro and multiple NPH injections. *Diabet Med*. 1999;16:319-324.
58. Renner R, Pfutzner A, Trautmann M, Harzar O, Sauter K, Landgraf R. Use of insulin lispro in continuous subcutaneous insulin infusion treatment. *Diabetes Care*. 1999;22:784-788.
59. Gale EA. A randomized, controlled trial comparing insulin lispro with human soluble insulin in patients with type 1 diabetes on intensified insulin therapy. *Diabet Med*. 2000;17:209-214.
60. Annuzzi G, Del Prato S, Arcari R, et al. Preprandial combination of lispro and NPH insulin improves overall blood glucose control in type 1 diabetic patients. *Nutr Metab Cardiovasc Dis*. 2001;11:168-175.
61. Murphy NP, Keane SM, Ong KK, Ford ME, Edge JA, Dunger BD. A randomized cross-over trial comparing insulin glargine plus lispro with NPH insulin plus soluble insulin in adolescents with type 1 diabetes. *Diabetes*. 2002;51(suppl 2):A54.
62. Lalli C, Ciofetta M, Del Sindaco P, et al. Long-term intensive treatment of type 1 diabetes with the short-acting insulin analog lispro in variable combination with NPH insulin at mealtime. *Diabetes Care*. 1999;22:468-477.
63. Raskin P, Klaff L, Bergenstal R, Halle JP, Donley D, Mecca T. A 16-week comparison of the novel insulin analog insulin glargine (HOE 901) and NPH human insulin used with insulin lispro in patients with type 1 diabetes. *Diabetes Care*. 2000;23:1666-1671.
64. Rosenstock J, Park G, Zimmerman J. Basal insulin glargine (HOE 901) versus NPH insulin in patients with type 1 diabetes on multiple daily insulin regimens. *Diabetes Care*. 2000;23:1137-1142.
65. Pieber TR, Eugene-Jolchine I, Derobert E. Efficacy and safety of HOE 901 versus NPH insulin in patients with type 1 diabetes. *Diabetes Care*. 2000;23: 157-162.
66. Witthaus E, Stewart J, Bradley C. Treatment satisfaction and psychological well-being with insulin glargine compared with NPH in patients with type 1 diabetes. *Diabet Med*. 2001;18:619-625.
67. Roach P, Strack T, Arora V, Zhao Z. Improved glycaemic control with the use of self-prepared mixtures of insulin lispro and insulin lispro protamine suspension in patients with types 1 and 2 diabetes. *Int J Clin Pract*. 2001;55:177-182.
68. Tamas G, Marre M, Astorga R, et al. Glycaemic control in type 1 diabetic patients using optimised insulin aspart or human insulin in a randomised multinational study. *Diabetes Res Clin Pract*. 2001;54:105-114.
69. Tsui E, Barnie A, Ross S, Parks R, Zinman B. Intensive insulin therapy with insulin lispro. *Diabetes Care*. 2001;24:1722-1727.
70. Bode BW, Strange P. Efficacy, safety, and pump compatibility of insulin aspart used in continuous subcutaneous insulin infusion therapy in patients with type 1 diabetes. *Diabetes Care*. 2001;24:69-72.
71. Rossetti P, Pampanelli S, Costa E, Torlone E, Brunetti P, Bolli G. A three-month comparison between multiple daily NPH and once daily glargine insulin administration in intensive replacement of basal insulin in type 1 diabetes mellitus. *Diabetes*. 2002;51(suppl 2):A54.
72. Boehm BO, et al. Premixed insulin aspart 30 vs premixed human insulin 30/70 twice daily. *Diabet Med*. 2002;19:393-399.
73. Seigler DE, Olsson GM, Skyler JS. Morning versus bedtime isophane insulin in type 2 (non-insulin dependent) diabetes mellitus. *Diabet Med*. 1992;9:826-833.
74. Groop LC, Widen E, Ekstrand A, et al. Morning or bedtime NPH insulin combined with sulfonylurea in treatment of NIDDM. *Diabetes Care*. 1992;15:831-834.
75. Taylor R, Davies R, Fox C, Sampson M, Weaver JU, Wood L. Appropriate insulin regimes for type 2 diabetes. *Diabetes Care*. 2000;23:1612-1618.
76. Tindall H, Bodansky HJ, Strickland M, Wales JK. A strategy for selection of elderly type 2 diabetic patients for insulin therapy, and a comparison of two insulin preparations. *Diabet Med*. 1988;5:533-536.
77. Paterson KR, Wilson M, Kesson CM, et al. Comparison of basal and prandial insulin therapy in patients with secondary failure of sulfonylurea therapy. *Diabet Med*. 1991;8:40-43.
78. Soneru IL, Agrawal L, Murphy JC, Lawrence AM, Abraira C. Comparison of morning or bedtime insulin with and without glyburide in secondary sulfonylurea failure. *Diabetes Care*. 1993;16:896-901.
79. Taylor R, Foster B, Kyne-Grzebalski D, Vanderpump M. Insulin regimens for the non-insulin dependent. *Diabet Med*. 1994;11:551-557.
80. Saudek CD, Duckwonk WC, Giobbie-Hurder A, et al. Implantable insulin pump vs multiple-dose insulin for non-insulin-dependent diabetes mellitus: a randomized clinical trial. *JAMA*. 1996;276:1322-1327.
81. Bastyr EJ 3rd, Stuart CA, Brodows RG, et al. Therapy focused on lowering postprandial glucose, not fasting glucose, may be superior for lowering HbA1c: IOEZ Study Group. *Diabetes Care*. 2000;23:1236-1241.
82. Bastyr EJ 3rd, Huang Y, Brunelle RL, Vignati L, Cox DJ, Kotsamos JG. Factors associated with nocturnal hypoglycaemia among patients with type 2 diabetes new to insulin therapy. *Diabetes Obes Metab*. 2000; 2:39-46.
83. Fritsche A, Schweitzer MA, Haring H. Improved glycemic control and reduced nocturnal hypoglycemia in patients with type 2 diabetes with morning administration of insulin glargine compared with NPH insulin. *Diabetes*. 2002;51(suppl 2):A54.
84. Lean ME, Ng LL, Tennison BR. Interval between insulin injection and eating in relation to blood glucose control in adult diabetics. *BMJ (Clin Res Ed)*. 1985; 290:105-108.
85. Sackey AH, Jefferson IG. Interval between insulin injection and breakfast in diabetes. *Arch Dis Child*. 1994;71:248-250.
86. Stades AM, Hoekstra JB, van der Tweed I, Erkelens DW, Holleman F, STABILITY Study Group. Additional lunchtime basal insulin during insulin lispro intensive therapy in a randomized, multicenter, crossover study in adults. *Diabetes Care*. 2002;25:712-717.
87. Tunbridge FK, et al. A comparison of human ultralente- and lente-based twice-daily injection regimens. *Diabet Med*. 1989;6:496-501.
88. Bolli GB, Owens DR. Insulin glargine. *Lancet*. 2000; 356:443-445.
89. Porcellati F, Rossetti P, Fanelli CG, Scionti L, Brunetti P, Bolli GB. Glargine vs NPH as basal insulin in intensive treatment of T1DM given lispro at meals. *Diabetes*. 2002;51(suppl 2):A53.
90. Kelly JL, Trence DL, Hirsch IB. Rapid decrease in clinically significant hypoglycemia with insulin glargine. *Diabetes*. 2002;51:A123.
91. Hayward RA, Manning WG, Kaplan SH, Wagner EH, Greenfield S. Starting insulin therapy in patients with type 2 diabetes. *JAMA*. 1997;278:1663-1669.
92. United Kingdom Prospective Diabetes Study 24: a 6-year, randomized, controlled trial comparing sulfonylurea, insulin, and metformin therapy in patients with newly diagnosed type 2 diabetes that could not be controlled with diet therapy. *Ann Intern Med*. 1998;128:165-175.
93. Wright A, Burden AC, Paisey RB, et al. Sulfonylurea inadequacy. *Diabetes Care*. 2002;25:330-336.
94. Turner RC, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus. *JAMA*. 1999;281:2005-2012.
95. Shichiri M, Kishikawa H, Ohkubo Y, Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care*. 2000;23(suppl 2):B21-B29.
96. Ohkubo Y, Kishikawa H, Arski E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract*. 1995;28:103-117.
97. Chow CC, Tsang LW, Sorensen JP, Cochram CS. Comparison of insulin with or without continuation of oral hypoglycemic agents in the treatment of secondary failure in NIDDM patients. *Diabetes Care*. 1995; 18:307-314.
98. van der Does FE, de Neeling JN, Snoek FJ, et al. Randomized study of two different target levels of glycemic control within the acceptable range in type 2 diabetes. *Diabetes Care*. 1998;21:2085-2093.
99. Dunbar JM, Madden PM, Gleeson DT, Fiad TM, McKenna TJ. Premixed insulin preparations in pen syringes maintain glycemic control and are preferred by patients. *Diabetes Care*. 1994;17:874-878.
100. Small M, Mac Rury S, Boal A, Peterson KR, Mac Cuish AC. Comparison of conventional twice daily subcutaneous insulin administration and a multiple injection regimen (using the NovoPen) in insulin-dependent diabetes mellitus. *Diabetes Res*. 1988;8:85-89.
101. Saurbrey N, Arnold-Larson S, Moller-Jensen B, Kuhl C. Comparison of continuous subcutaneous insulin infusion with multiple insulin injections using the NovoPen. *Diabet Med*. 1988;5:150-153.

102. Murray DP, Keenan P, Gayer E, et al. A randomized trial of the efficacy and acceptability of a pen injector. *Diabet Med.* 1988;5:750-754.
103. Chen HS, Hwu CM, Kwok CF, et al. Clinical response and patient acceptance of a prefilled, disposable insulin pen injector for insulin-treated diabetes. *Zhonghua Yi Xue Za Zhi (Taipei).* 1999;62:455-460.
104. Groop L, Harno K, Tolppanen EM. The combination of insulin and sulfonylurea in the treatment of secondary drug failure in patients with type II diabetes. *Acta Endocrinol (Copenh).* 1984;106:97-101.
105. Groop L, Hano K, Nikkila EA, Pelkonan R, Tolppanen EM. Transient effect of the combination of insulin and sulfonylurea (glibenclamide) on glycemic control in non-insulin dependent diabetics poorly controlled with insulin alone. *Acta Med Scand.* 1985;217:33-39.
106. Samanta A, Burden AC, Kinghorn HA. A comparative study of sulfonylurea and insulin therapy in non insulin dependent diabetics who had failed on diet therapy alone. *Diabetes Res.* 1987;4:183-185.
107. Schade DS, Mitchell WJ, Griego G. Addition of sulfonylurea to insulin treatment in poorly controlled type II diabetes. *JAMA.* 1987;257:2441-2445.
108. Kitabchi AE, Soria AG, Radparvar A, Lawson-Grant V. Combined therapy of insulin and tolazamide decreases insulin requirement and serum triglycerides in obese patients with noninsulin-dependent diabetes mellitus. *Am J Med Sci.* 1987;294:10-14.
109. Holman RR, Steemson J, Turner RC. Sulphonylurea failure in type 2 diabetes. *Diabet Med.* 1987;4:457-462.
110. Stenman S, Groop PH, Saloranta C, Totterman KJ, Fyhrqvist F, Groop L. Effects of the combination of insulin and glibenclamide in type 2 (non-insulin-dependent) diabetic patients with secondary failure to oral hypoglycaemic agents. *Diabetologia.* 1988;31:206-213.
111. Lewitt MS, Yu VK, Rennie GC, et al. Effects of combined insulin-sulfonylurea therapy in type II patients. *Diabetes Care.* 1989;12:379-383.
112. Riddle MC, Hart JS, Bouma DJ, Phillipson BE, Youker G. Efficacy of bedtime NPH insulin with daytime sulfonylurea for subpopulation of type II diabetic subjects. *Diabetes Care.* 1989;12:623-629.
113. Vigneri R, Trischitta V, Italia S, Mazzanno S, Rebuazzo MA, Squatrito S. Treatment of NIDDM patients with secondary failure to glyburide. *Diabet Metab.* 1991;17(1 pt 2):232-234.
114. Trischitta V, Italia S, Mazzarino S, et al. Comparison of combined therapies in treatment of secondary failure to glyburide. *Diabetes Care.* 1992;15:539-542.
115. Ravnik-Oblak M, Mrevlje F. Insulin versus a combination of insulin and sulfonylurea in the treatment of NIDDM patients with secondary oral failure. *Diabetes Res Clin Pract.* 1995;30:27-35.
116. Feinglos MN, Thacker CR, Lobaugh B, et al. Combination insulin and sulfonylurea therapy in insulin-requiring type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 1998;39:193-199.
117. Robinson AC, Burke J, Robinson S, Johnston DG, Elkeles RS. The effects of metformin on glycemic control and serum lipids in insulin-treated NIDDM patients with suboptimal metabolic control. *Diabetes Care.* 1998;21:701-705.
118. Lopez-Alvarenga JC, Aguilar-Salinas CA, Velasco-Perez ML, et al. Acarbose vs bedtime NPH insulin in the treatment of secondary failures to sulfonylurea-metformin therapy in type 2 diabetes mellitus. *Diabetes Obes Metab.* 1999;1:29-35.
119. Fritsche A, Schmulling RM, Haring HU, Stumvoll M. Intensive insulin therapy combined with metformin in obese type 2 diabetic patients. *Acta Diabetol.* 2000;37:13-18.
120. Ponssen HH, Elte JW, Wheat P, Schouten JP, Bets D. Combined metformin and insulin therapy for patients with type 2 diabetes mellitus. *Clin Ther.* 2000;22:709-718.
121. Osei K, O'Dorisio TM, Falko JM. Concomitant insulin and sulfonylurea therapy in patients with type II diabetes. *Am J Med.* 1984;77:1002-1009.
122. Falko JM, Osei K. Combination insulin/glyburide therapy in type II diabetes mellitus. *Am J Med.* 1985;79:92-101.
123. Quatraro A, Consoli G, Cariello A, Giugliano D. Combined insulin and sulfonylurea therapy in non-insulin-dependent diabetics with secondary failure to oral drugs: a one year follow-up. *Diabet Metab.* 1986;12:315-318.
124. Mauerhoff T, Ketelslegers JM, Lambert AE. Effect of glibenclamide in insulin-treated diabetic patients with a residual insulin secretion. *Diabet Metab.* 1986;12:34-38.
125. Gutniak M, Karlander SG, Efendic S. Glyburide decreases insulin requirement, increases beta-cell response to mixed meal, and does not affect insulin sensitivity. *Diabetes Care.* 1987;10:545-554.
126. Reich A, Abraira C, Lawrence AM. Combined glyburide and insulin therapy in type II diabetes. *Diabetes Res.* 1987;6:99-104.
127. Bachmann W, Lotz N, Mehnert H, Rosak C, Schoffling K. [Effectiveness of combined treatment with glibenclamide and insulin in secondary sulfonylurea failure: a controlled multicenter double-blind clinical trial]. *Dtsch Med Wochenschr.* 1988;113:631-636.
128. Casner PR. Insulin-glyburide combination therapy for non-insulin-dependent diabetes mellitus. *Clin Pharmacol Ther.* 1988;44:594-603.
129. Lawrence AM, Abraira C. New modalities in diabetes treatment. *Am J Med.* 1988;85:153-158.
130. Lins PE, Lundblads S, Perrson-Trotzig E, Adamson U. Glibenclamide improves the response to insulin treatment in non-insulin-dependent diabetics with second failure to sulfonylurea therapy. *Acta Med Scand.* 1988;223:171-179.
131. Klein W. Sulfonylurea-metformin-combination versus sulfonylurea-insulin-combination in secondary failures of sulfonylurea monotherapy. *Diabet Metab.* 1991;17(1 pt 2):235-240.
132. Groop L, Widen E. Treatment strategies for secondary sulfonylurea failure. *Diabet Metab.* 1991;17(1 pt 2):218-223.
133. Lebovitz HE. Insulin secretagogues: old and new. *Diabetes Rev.* 1999;7:139-153.
134. Riddle M, Hart J, Bingham P, Garrison C, McDaniel P. Combined therapy for obese type 2 diabetes. *Am J Med Sci.* 1992;303:151-156.
135. Chiasson JL, Josse RG, Hunt JA, et al. The efficacy of acarbose in the treatment of patients with non-insulin-dependent diabetes mellitus. *Ann Intern Med.* 1994;121:928-935.
136. Shank ML, Del Prato S, DeFronzo RA. Bedtime insulin/daytime glipizide. *Diabetes.* 1995;44:165-172.
137. Clauson P, Karlander S, Steen L, Effendic S. Daytime glibenclamide and bedtime NPH insulin compared to intensive insulin treatment in secondary sulfonylurea failure. *Diabet Med.* 1996;13:471-477.
138. Wolffenbuttel BH, Sels JP, Rondas-Colbeas GJ, Menheere PP, Nieuwanhuijzen Krusaman AC. Comparison of different insulin regimens in elderly patients with NIDDM. *Diabetes Care.* 1996;19:1326-1332.
139. Colwell JA. The feasibility of intensive insulin management in non-insulin-dependent diabetes mellitus. *Ann Intern Med.* 1996;124(1 pt 2):131-135.
140. Relimpio F, Pumer A, Losada F, Mangas MA, Acosta D, Astorga R. Adding metformin versus insulin dose increase in insulin-treated but poorly controlled type 2 diabetes mellitus. *Diabet Med.* 1998;15:997-1002.
141. Niazi R, Muzaffar Z. Comparison of bedtime NPH insulin or metformin combined with glibenclamide in secondary sulfonylurea failure in obese type II (NIDDM) patients. *J Pak Med Assoc.* 1998;48:336-338.
142. Kelley DE, Bidot P, Freidman Z, et al. Efficacy and safety of acarbose in insulin-treated patients with type 2 diabetes. *Diabetes Care.* 1998;21:2056-2061.
143. Riddle MC, Schneider J. Beginning insulin treatment of obese patients with evening 70/30 insulin plus glimepiride versus insulin alone. *Diabetes Care.* 1998;21:1052-1057.
144. Yki-Jarvinen H, Ruysy L, Nikkila K, Tulokas T, Vanamo R, Heikkilla M. Comparison of bedtime insulin regimens in patients with type 2 diabetes mellitus. *Ann Intern Med.* 1999;130:389-396.
145. Aviles-Santa L, Sinding J, Raskin P. Effects of metformin in patients with poorly controlled, insulin-treated type 2 diabetes mellitus. *Ann Intern Med.* 1999;131:182-188.
146. Bastyr EJ 3rd, Johnston ME, Trautmann ME, Anderson JH Jr, Vignati L. Insulin lispro in the treatment of patients with type 2 diabetes mellitus after oral agent failure. *Clin Ther.* 1999;21:1703-1714.
147. Wulffele MG, Kooy A, Lehert P, et al. Discontinuation of metformin in type 2 diabetes patients treated with insulin. *Neth J Med.* 2002;60:249-252.
148. Yki-Jarvinen H. Combination therapies with insulin in type 2 diabetes. *Diabetes Care.* 2001;24:758-767.
149. Frazier LM, Malrow CD, Alexander LT Jr, et al. Need for insulin therapy in type II diabetes mellitus. *Arch Intern Med.* 1987;147:1085-1089.
150. Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes: scientific review. *JAMA.* 2002;287:360-372.
151. Strowig SM, Aviles-Santa ML, Raskin P. Comparison of insulin monotherapy and combination therapy with insulin and metformin or insulin and troglitazone in type 2 diabetes. *Diabetes Care.* 2002;25:1691-1698.
152. Delea T, Hagiwara M, Edelsberg J, et al. Exposure to glitazone antidiabetics and risk of heart failure among persons with type 2 diabetes. *J Am Coll Cardiol.* 2002;39(suppl A):184A.
153. Abraira C, Colwell JA, Nuttall FQ, et al. Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). *Diabetes Care.* 1995;18:1113-1123.
154. Henry RR, Gumbiner B, Ditzler T, Wallace P, Lyon R, Glauber NS. Intensive conventional insulin therapy for type II diabetes. *Diabetes Care.* 1993;16:21-31.
155. Pickup J, Mattock M, Kerry S. Glycaemic control with continuous subcutaneous insulin infusion compared with intensive insulin injections in patients with type 1 diabetes. *BMJ.* 2002;324:705.
156. Gabbe SG, Holing E, Temple P, Brown ZA. Benefits, risks, costs, and patient satisfaction associated with insulin pump therapy for the pregnancy complicated by type 1 diabetes mellitus. *Am J Obstet Gynecol.* 2000;182:1283-1291.
157. Thompson DM, Kozak SE, Sheps S. Insulin adjustment by a diabetes nurse educator improves glucose control in insulin-requiring diabetic patients. *CMAJ.* 1999;161:959-962.
158. Biermann E, Dietrich W, Standl E. Telecare of diabetic patients with intensified insulin therapy. *Stud Health Technol Inform.* 2000;77:327-332.
159. Peters A, Rubsamen M, Jacob U, Look D, Scriba PC. Clinical evaluation of decision support system for insulin-dose adjustment in IDDM. *Diabetes Care.* 1991;14:875-880.
160. Schrezenmeir J, Dirting K, Papazov P. Controlled multicenter study on the effect of computer assistance in intensive insulin therapy of type 1 diabetics. *Comput Methods Programs Biomed.* 2002;69:97-114.
161. Holmboe ES. Oral antihyperglycemic therapy for type 2 diabetes: clinical applications. *JAMA.* 2002;287:373-376.
162. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with sulfonylurea monotherapy in type 2 diabetes. *Diabetes Care.* 2002;25:2244-2248.
163. *Practical Insulin: A Handbook for Prescribing Providers.* Alexandria, Va: American Diabetes Association; 2002.

eTable 1. Randomized Controlled Trials of Standard Human Insulins vs Currently Available Insulin Analogues in Type 1 Diabetes Mellitus

Source	No. of Patients	Study Length*	Treatment Arms†	Main Outcomes (Glucose Reported as mg/dL)‡
			Crossover Trials	
Pfutzner et al,[54] 1996	107	3 mo	Lispro/NPH vs regular/NPH	No difference in HbA_{1c} levels between groups but fewer hypoglycemic events with lispro (absolute RR, 6%; $P = .008$)
Daniels et al,[51] 1997	20	3 mo	Lispro vs regular with basal NPH, NPL, or Ultralente	No differences in HbA_{1c} levels or hypoglycemia between groups
Zinman et al,[52] 1997	30	3 mo	Lispro vs regular in CSII	Double-blind trial HbA_{1c} levels lower with lispro ($P = .0041$) Lispro lowered PPBG levels after all meals (breakfast, $P = .006$; lunch, $P = .049$; dinner, $P = .03$) No difference in hypoglycemia between groups
Vignati et al,[53] 1997	379	2 mo	Twice-daily NPH/prandial regular vs lispro	Treatment to target FPG level <140 and PPPG level <160 Lispro lowered PPBG levels (breakfast, $P<.001$; dinner, $P<.056$) No difference in HbA_{1c} levels or hypoglycemia between groups
Del Sindaco et al,[54] 1998	69	3 mo	Lispro/bedtime NPH vs regular/bedtime NPH	Group 1, lispro with bedtime NPH showed no difference in HbA_{1c} levels but an increase in hypoglycemic episodes ($P<.05$) Group 2, lispro with NPH 3-4 times/d showed a decrease in HbA_{1c} levels with no increase in hypoglycemic events Group 3, regular at meals with no lag time with bedtime NPH showed no change in HbA_{1c} levels or hypoglycemic episodes Group 4, regular with a lag time of 10-40 minutes with bedtime NPH showed higher HbA_{1c} levels without lag time (group 3) but with an increase in hypoglycemic events with longer lag times
Home et al,[55] 1998	90 men	4 wk	Aspart/bedtime NPH vs regular/bedtime NPH	No changes in fructosamine levels (short HbA_{1c} equivalent) Aspart improved PPG control after lunch and dinner ($P<.05$) Regular lowered nighttime glucose levels ($P<.01$) Severe hypoglycemic episodes decreased with aspart (absolute RR, 38%; $P<.002$)
Melki et al,[56] 1998	39	3 mo	Lispro vs regular in CSII	HbA_{1c} levels were lower with lispro ($P = .01$) PPBG levels were lower with lispro ($P<.001$) as were SDs of all BG levels ($P<.02$) No differences in hypoglycemic events between groups 95% of patients chose to continue lispro
Colombel et al,[57] 1999	25	3 mo	Lispro/NPH vs regular/NPH	Variability in BG levels was lower with lispro ($P<.01$) but no change in HbA_{1c} levels Less severe hypoglycemic events were observed with lispro ($P = .048$; absolute RR, 45%)
Renner et al,[58] 1999	113	4 mo	Lispro vs regular in CSII	HbA_{1c} levels were significantly better ($P = .02$) and PPBG levels better after all meals ($P<.001$) with lispro No difference in hypoglycemic events between groups Patient satisfaction was higher with lispro
Gale,[59] 2000	93	12 wk	Lispro/bedtime NPH vs regular/bedtime NPH	No difference in HbA_{1c} levels between groups Decrease in nocturnal hypoglycemic episodes with lispro (absolute RR, 44%; $P<.001$) Increase in hypoglycemic episodes with lispro between 6 AM and noon (absolute RI, 12%; $P = .03$)
Annuzzi et al,[60] 2001	85	3 mo	Regular/NPH vs lispro/NPH	Basal NPH at evening with prandial regular or lispro NPH could be added at breakfast and lunch if necessary Number of injections was kept constant: 42% of patients injected 3 times/d and 58% of patients injected 4 times/d FBG and preprandial BG levels were similar PPBG levels improved with insulin lispro at all 3 meals ($P = .003$) HbA_{1c} levels improved with lispro (8.1% vs 8.3%; $P<.05$) No difference in hypoglycemia between groups Better rate of patient acceptance of lispro ($P<.001$)
Ferguson et al,[61] 2001	33	24 wk	Lispro/NPH vs regular/NPH	Patients with known hypoglycemia unawareness who had HbA_{1c} levels of approximately 9%, and 55% of patients had 1 or more severe hypoglycemic episodes Fewer severe hypoglycemic episodes with lispro (55% vs 84%; $P = .09$; absolute RR = 20%)
Raskin et al,[62] 2001	58	12 wk; 2-way	Lispro vs buffered regular in CSII	Open-label study Lower HbA_{1c} levels with lispro ($P = .004$) FPG levels were the same but PPBG levels at 1 hour ($P = .012$) and 2 hours ($P = .001$) were lower with lispro No difference in hypoglycemia between groups
Murphy et al,[63] 2002	25	16 wk	Lispro/glargine vs regular/bedtime NPH	Glargine produced lower FBG levels (144 vs 166; $P<.0001$) and lower PPBG levels ($P<.005$) Nocturnal hypoglycemic events were less with glargine ($P<.05$) HbA_{1c} levels were lower (8.7% vs 9.1%; not significant) despite lower insulin doses (1.16 vs 1.26 U/kg; $P<.005$) with glargine and lispro

(continued)

eTable 1. Randomized Controlled Trials of Standard Insulins (NPH and Regular) vs Currently Available Insulin Analogues in Type 1 Diabetes Mellitus (cont)

Source	No. of Patients	Study Length*	Treatment Arms	Main Outcomes (Glucose Reported as mg/dL)
			Parallel Trials	
Lalli et al,[58] 1999	56	1 y	Lispro/bedtime NPH vs regular/bedtime NPH	Lispro taken at meals; regular taken at meals with 10-40 minute lag time Small doses of basal NPH at mealtime with lispro In lispro group, mean BG levels were lower ($P<.05$), HbA_{1c} levels were lower ($P<.002$), less frequent hypoglycemic episodes (absolute RR, 22%; $P<.05$)
Raskin et al,[63] 2000	619	16 wk	Prandial lispro/glargine vs prandial lispro/NPH	Decreased FBG levels ($P = .001$); more people in glargine (30%) than in NPH (17%) group reached FBG target level (n = 119) No differences in HbA_{1c} levels or hypoglycemia between groups Weight gain of 0.54 kg in NPH group vs 0.12 kg in glargine ($P = .034$) group
Rosenstock et al,[61] 2000	256	4 wk	Prandial regular/glargine vs prandial regular/NPH regular	FPG levels decreased by 40 for glargine compared with NPH Glargine was superior to NPH in reducing FPG levels in patients previously receiving twice-daily NPH but not in those previously taking bedtime NPH
Ratner et al,[62] 2000	534	28 wk	Prandial regular/glargine vs prandial regular/NPH	No changes in HbA_{1c} levels but a significant reduction in FPG levels with glargine ($P = .0145$) Fewer hypoglycemic episodes (absolute RR, 27%; $P = .02$) and fewer nocturnal hypoglycemic episodes (absolute RR, 22%; $P = .01$) with glargine
Pieber et al,[56] 2000	333	4 wk	Prandial regular/glargine vs prandial regular/NPH	FPG and FBG levels were lower ($P<.005$) with glargine Glargine lowered HbA_{1c} levels ($P = .03$) and decreased frequency of nocturnal hypoglycemic episodes (36% vs 55%; absolute RR, 19%; $P = .004$, significant compared with once- but not twice-daily NPH)
Home et al,[59] 2000	1070	6 mo	Aspart/NPH vs regular/NPH	Aspart reduced HbA_{1c} levels (0.12%; 95% CI, 0.03-0.22; $P<.02$) Lower PPBG levels ($P<.01$) but higher preprandial levels before breakfast and dinner ($P<.01$) Patient satisfaction better with aspart ($P<.001$) Fewer nocturnal hypoglycemic events (absolute RR, 15%; $P<.05$) with aspart Absolute RR for severe hypoglycemic events was 9% for aspart
Witthaus et al,[60] 2001	517	28 wk	Glargine/prandial regular vs NPH/prandial regular	Quality-of-life questionnaires showed glargine significantly improved treatment satisfaction with lower perceived frequency of hypoglycemic episodes ($P = .002$) No difference in psychological well being between groups
Roach et al,[57] 2001	166 (102 type 1 DM; 64 type 2 DM)	12 mo	Freemix 75% NPL/25% lispro vs freemix NPH/regular	BG levels were lower with lispro/NPL 2 hours after breakfast ($P = .001$), before lunch ($P = .016$), 2 hours after evening meal ($P<.001$), and at bedtime ($P = .001$) at end point No differences in HbA_{1c} levels between groups Authors of Scientific Review note that physicians and patients were allowed to adjust insulin freely after 3 months with NPH, regular, or lispro, making this study difficult to interpret
Tamas et al,[65] 2001	423	3 mo	Aspart/NPH vs regular/NPH	HbA_{1c} levels were lower with aspart ($P<.05$) and PPBG levels were lower after breakfast ($P<.001$) and dinner ($P<.01$) No difference in hypoglycemia between groups Patient(s) satisfaction for perceived hypoglycemia was improved with aspart ($P = .005$) and patients perceived increased flexibility ($P = .002$)
Tsui et al,[69] 2001	27	9 mo	Lispro with basal NPH vs lispro in CSII	No difference in HbA_{1c} levels, in hypoglycemia, and in quality-of-life scores between groups
Bode and Strange,[70] 2001	29	7 wk	Aspart vs regular in CSII	Open-label study for aspart (n = 19) vs buffered regular (n = 10) insulin No differences in HbA_{1c} levels between groups Fewer hypoglycemic events with aspart (absolute RR, 6%)
Roach et al,[67] 2001	102	12 mo	Freemix lispro/NPL vs regular/NPH	Lower BG levels at multiple points for the freemix lispro group HbA_{1c} levels lower with NPL/L (7.54% vs 7.2%; $P = .02$) with similar insulin doses No difference in hypoglycemia between groups
Rossetti et al,[71] 2002	51	3 mo	Once-daily glargine/lispro vs 4 times/d NPH/lispro	Mean BG level was lower with glargine vs NPH (134 vs 149; $P<.05$) HbA_{1c} levels decreased with glargine (−0.4% to 6.5%; $P<.05$ vs NPH) Use of glargine increased basal and decreased prandial insulin vs NPH ($P<.05$) Fewer mild hypoglycemic events with glargine (approximately 7.9 vs 12.2 events per patient per month; absolute RR, 21%; $P<.05$) No significant difference between dinner or bedtime glargine
Boehm et al,[72] 2002	104	12 wk	Twice-daily 70% protamine aspart/30% aspart (BIAsp) vs twice-daily 70% NPH/30% regular	BIAsp lowered all CBG levels (postbreakfast, prelunch, postdinner, and bedtime were lower by approximately 18, $P<.05$) HbA_{1c} level was 0.1% lower (not significant) with 0.3 U/kg (0.65 vs 0.62 U/kg) mean increase insulin total dose per day in the BIAsp group ($P<.01$) but no weight gain Severe hypoglycemic episodes were reduced by 50% (14 vs 30) in the BIAsp group

Abbreviations: BG, blood glucose; BIAsp, biphasic insulin aspart; CI, confidence interval; CSII, continuous subcutaneous insulin infusion; DM, diabetes mellitus; FBG, fasting (whole) blood glucose (usually capillary blood glucose); FPG, fasting plasma glucose; Hb, hemoglobin; NPH, isophane insulin (neutral protamine Hagedorn); NPL, insulin lispro protamine (neutral protamine lispro); PP, postprandial; RR, risk reduction; RI, risk increase.

SI conversion factor: for glucose, to convert mg/dL to mmol/L, multiply by 0.0555.

*Crossover study time is months in each arm.

†Ultralente is insulin zinc extended.

‡Authors of Scientific Review comment on study interpretation: When regular insulin vs lispro or aspart insulin is used with NPH as a basal/prandial insulin, NPH confuses the ability to separate outcomes by insulin type and essentially flaws these studies.

eTable 2. Randomized Controlled Trials Comparing Insulin Regimens in Type 2 Diabetes Mellitus

Source	No. of Patients	Study Length*	Treatment Arms†	Main Outcomes (Glucose Reported as mg/dL)
			Crossover Trials	
Seigler et al,[73] 1992	12	4 mo	Morning vs bedtime NPH	FPG levels better with bedtime insulin ($P<.001$) Mean HbA_{1c} levels better with bedtime NPH (6.2% vs 5.8%; $P<.05$) with no difference in insulin dose
Groop et al,[74] 1992	24	3 mo	Morning NPH vs bedtime NPH	Glibenclamide control, and continued in both arms Similar treatment effects and no difference in HbA_{1c} levels between groups Bedtime NPH led to lower FBG levels ($P<.01$) and NPH at morning led to lower evening BG levels ($P<.01$)
Vignati et al,[53] 1997	328	2 mo	Twice daily NPH/premeal regular vs NPH/lispro	Treatment to target FPG level <140 and PPPG level <160 Lispro lowered PPBG levels (breakfast, $P<.001$ and dinner, $P<.056$) No difference in HbA_{1c} levels or episodes of hypoglycemia between groups
Taylor et al,[75] 2000	79	6 mo	Once-daily Ultralente vs twice-daily NPH	HbA_{1c} levels lower with NPH use (~9.0% vs ~9.7%; $P<.01$) More severe hypoglycemic episodes with Ultralente ($P<.01$) Quality of life improved in both groups but improved more with NPH ($P<.001$)
			Parallel Trials	
Tindall et al,[76] 1988	22	6 mo	Humulin-Zn vs Neulente	Older patients (aged 48-88 y) HbA_{1c} levels improved significantly from baseline (13.2% to 10.6% with Humulin-Zn and to 11.2% with Neulente) vs 46 patients who continued taking oral agents Fewer hypoglycemic episodes in the Neulente group (4 vs 46) 6 Humulin-Zn patients and 1 Neulente patient required short-acting insulin to overcome high PPBG levels
Paterson et al,[77] 1991	35	14 wk	Basal Ultralente vs prandial regular	Evening Ultralente vs 3 premeal regular insulin injections with 15-minute lag time HbA_{1c} levels improved (from 12.5% to 10.7% for basal and from 12.0% to 9.5% for prandial) FPG levels did not differ between groups and insulin dose was higher in prandial regular group (44 vs 27 U, $P<.005$) No difference in frequency or severity of hypoglycemic episodes between groups Weight gain was 2.7 kg in prandial regular group
Jennings et al,[27] 1991	20	4 mo	CSII regular vs twice-daily NPH/regular	8 of 10 CSII patients had HbA_{1c} levels <10.1% vs 3 of 10 taking NPH/regular Weight gain, hypoglycemia, and insulin dose were similar between groups
Soneru et al,[78] 1993	29	12-wk parallel and 6-wk insulin only	Glyburide and either morning or bedtime Lente then morning Lente vs bedtime Lente	Insulin adjusted to FPG target during 12-week combined, then glyburide stopped and insulin doses adjusted FPG levels same with morning or evening insulin with or without glyburide with FPG levels less than baseline ($P<.02$) No difference in HbA_{1c} levels and no change in lipid levels More hypoglycemic reactions with morning insulin (1.4 vs 0.4; $P<.025$) Weight gain of 3.0 kg during combination phase and did not increase during the insulin phase only
Taylor et al,[79] 1994	21	6 mo	Twice-daily intermediate-acting insulin vs prandial regular	HbA_{1c} levels improved similarly between groups PP control better with regular insulin use and FBG levels better with intermediate-acting insulin
Landstedt-Hallin et al,[72] 1995	80	16 wk	Glibenclamide/ bedtime NPH vs glibenclamide/ premeal regular	FBG levels were lower in NPH group (−115 vs −50; $P<.001$) HbA_{1c} levels and insulin doses were similar between groups Both insulin regimens lowered total cholesterol and triglyceride levels compared with baseline (all $P<.05$) Weight gain in regular insulin group was greater than NPH group (3.4 vs 1.9 kg; $P<.002$)
Saudek et al,[80] 1996	121	1 y	IIP vs MDI	HbA_{1c} levels improved similarly in both groups IIP reduced BG fluctuations and reduced mild hypoglycemic episodes by 68% ($P<.001$) IIP produced no weight gain and had better quality of life ($P = .03$)
Bastyr et al,[81] 2000‡	135	3 mo	Glyburide with either prandial lispro vs NPH bedtime vs MET	HbA_{1c} levels for all groups lower than baseline ($P<.001$), but lispro group (7.68%) was lower than NPH group (8.51%; $P = .003$) and MET group (8.31%; $P = .025$) FPG levels in NPH group (153) were lower than lispro group (190; $P = .001$) and MET group (174; $P = .029$) 2-Hour PPBG level in lispro group (196) was lower than NPH group (220; $P = .052$) and MET group (229; $P = .009$) No difference in hypoglycemic episodes between both groups ($P = .16$) More weight gain in insulin group than MET group ($P<.01$) and more in NPH group than lispro group ($P = .051$)

(continued)

eTable 2. Randomized Controlled Trials Comparing Insulin Regimens in Type 2 Diabetes Mellitus (cont)

Source	No. of Patients	Study Length*	Treatment Arms	Main Outcomes (Glucose Reported as mg/dL)
			Parallel Trials (cont)	
Bastyr et al,[35] 2000	365	12 mo	Basal NPH or Ultralente with either prandial regular vs lispro	Trend toward more nocturnal hypoglycemic episodes with regular (RR, 1.3; $P = .07$) and less noctural hypoglycemic episodes in a subset of 195 North American patients (RR = 1.6; $P<.01$) than with lispro More hypoglycemic episodes with Ultralente basal (RR = 1.5; $P = .02$) than with NPH
Yki-Jarvinen et al,[41] 2000	426	12 mo	Bedtime NPH vs glargine	Oral agents continued Treatment to target FBG level of 120 HbA_{1c} levels improved in both groups (8.2% vs 8.3%; $P<.001$) Fewer nocturnal hypoglycemic episodes with glargine (9.9% vs 24%; $P<.02$) Lower postdinner CBG levels with glargine (178 vs 193; $P<.02$)
Roach et al,[67] 2001	64	12 mo	Freemix lispro/NPL vs regular/NPH	Lower BG levels at multiple points Lispro/NPH lowered HbA_{1c} levels by 0.4%: 7.54% ($P = .02$) with similar insulin doses No difference in hypoglycemia between groups Authors of Scientific Review note that NPL is not sold separately but it is functionally identical to NPH
Rosenstock et al,[24] 2001	518	28 wk	Glargine bedtime vs once- or twice-daily NPH	Dose adjustment to FBG target level <120 63% took prandial regular insulin Both arms reduced HbA_{1c} levels (−0.41 to −0.59%, with HbA_{1c} level approximately 8.5%; $P<.001$) and FBG level ($P<.001$) vs baseline Less nocturnal hypoglycemic episodes (26.5% vs 35.5%; $P = .014$) and weight gain (0.4 vs 1.4 kg; $P<.007$) with glargine
Fritsche et al,[43] 2002	695	24 wk	Glimepiride with morning glargine or bedtime glargine or bedtime NPH	Insulin titrated to FBG target level <101 HbA_{1c} level improved in all groups vs baseline (morning glargine, −1.29% vs bedtime glargine, −1.01%; $P = .009$) More achieved HbA_{1c} level <8% with morning (58%; $P = .002$) or bedtime glargine (54%; $P = .046$) than NPH (44%) Lowest rate of hypoglycemic episodes with morning glargine vs NPH (17% vs 38%; $P = .001$) Authors of Scientific Review note that doses not specified; morning vs evening glargine may differ based on actual amount of insulin available for meals and timing of hypoglycemia
Boehm et al,[72] 2002	187	12 wk	Twice-daily 70% protamine aspart/30% aspart (BIAsp) vs twice-daily 70% NPH/30% regular	BIAsp lowered all BG levels (postbreakfast, prelunch, postdinner, bedtime) with a mean approximately 18 Severe hypoglycemic episodes were reduced by 50% (6 vs 12) in the BIAsp group

Abbreviations: BG, blood glucose; BIAsp, biphasic insulin aspart; CSII, continuous subcutaneous insulin infusion; FBG, fasting blood glucose; Hb, hemoglobin; IIP, implantable insulin pump; MDI, multiple daily (insulin) injections; MET, metformin; NPH, isophane insulin (neutral protamine Hagedorn); NPL, insulin lispro protamine (neutral protamine lispro); PP, postprandial; RR, relative risk.

SI conversion factor: for glucose, to convert mg/dL to mmol/L, multiply by 0.0555.

*Crossover study time is time in each arm.

†Ultralente is insulin zinc extended and Lente is insulin zinc.

‡Study also included an oral agent arm but this study is not included in eTable 3.

eTable 3. Randomized Controlled Trials of Combination Therapy With Available Oral Agents and Human or Animal Insulin

Source	No. of Patients	Study Length	Treatment Arms*	Outcomes (Glucose Reported as mg/dL)†
			Crossover Trials	
Groop et al,[104] 1984	13	2 mo	Insulin/glibenclamide vs insulin/placebo	Insulin and glibenclamide lowered FPG levels (P = .026) and increased C-peptide levels (P = .037) vs placebo
Groop et al,[105] 1985	13	8 wk	Insulin/glibenclamide vs insulin/placebo	Combination therapy lowered FBG levels (P<.001) and HbA_{1c} levels (P<.05), and increased C-peptide levels (P<.01) No difference in insulin dose, weight gain, or lipid levels between groups
Samanta et al,[106] 1987	20	3 mo	NPH/regular vs tolbutamide	Insulin lowered PPBG levels (139 vs 157; P<.05) and HbA_{1c} levels (6.6% vs 7.8%; P<.05) more than with SU C-peptide levels improved after insulin therapy (P<.05)
Schade et al,[107] 1987	16	16 wk	Once- or twice-daily insulin (NPH or Lente) and either glyburide or placebo	Insulin dose could only be decreased HbA_{1c} levels were lower with SU therapy (10.2% vs 10.9%; P<.05) with lower insulin dose (53.5 vs 54.9 U; P<.05) Similar weight gain between groups
Kitabchi et al,[108] 1987	12	3 mo	NPH vs NPH/tolbutamide	As C-peptide levels increased to 70% (P<.02) with similar glucose controls, combined therapy lowered insulin dose 23% (0.69 vs 0.89 U/kg; P<.002) and triglyceride levels (142 vs 181 mg/dL) (P<.05)
Holman et al,[109] 1987	15	8-wk multiple crossover trial	Control (SU) vs SU/MET, or Ultralente, or SU/Ultralente, or Ultralente/regular	HbA_{1c} level for control SU was 10.7% HbA_{1c} level for Ultralente was 10.1% (P = .002) HbA_{1c} level for SU and Ultralente was 9.5% (P<.001) HbA_{1c} level for Ultralente and regular was 9.4% (P = .001) SU and Ultralente showed no improvement over Ultralente alone, but less insulin was used (25 vs 40 U; P = .001)
Stenman et al,[110] 1988	15	4 mo	Insulin/glibenclamide vs insulin/placebo	Insulin and SU lowered HbA_{1c} levels (8.3% vs 9.1%; P<.001) and FPG levels (133 vs 164; P<.05) with lower insulin dose (−10 U; P<.001), but with increased frequency of hypoglycemia (P<.01) and at a 30% to 50% higher cost
Woffenbuttel et al,[35] 1989	13	6 mo	Insulin vs SU	HbA_{1c} levels lower with insulin vs SU (9.5% vs 11.0%; P<.05) Insulin produced greater weight gain (4.2 vs 1.1 kg; P<.05) HDL levels higher and triglyceride levels lower with insulin (P<.05)
Lewitt et al,[111] 1989	31	12 wk	Insulin/glyburide vs insulin/placebo	Insulin and glyburide improved HbA_{1c} levels from 9.9% to 9.1% (P<.001) Responders had higher C-peptide levels and shorter duration of disease predicted response to SU
Riddle et al,[112] 1989	20	4 mo	Bedtime insulin/glyburide vs insulin/placebo	FPG levels (P<.01) and HbA_{1c} levels (9.8% vs 10.6%; P<.01) improved in the SU group More weight gain insulin and glyburide therapy
Vignen et al,[113] 1991	12	8 wk	Glyburide/bedtime NPH vs glyburide/MET	PPBG levels were lower with MET (196 vs 249; P<.05) Increase in weight gain with NPH (2 kg; P<.005) No difference in FPG or HbA_{1c} levels between groups
Trischitta et al,[114] 1992	16	8 wk	Glyburide/bedtime NPH vs glyburide/MET	NPH dose fixed at 0.2 U/kg No difference in HbA_{1c} levels between groups PPPG level of 239 for NPH vs 203 for MET (P<.05) More weight gain with NPH (1.9 kg; P<.01)
Ravnik-Oblak and Mrevlje,[115] 1995	27	3-mo crossover + 1 y	NPH/regular/glibenclamide vs NPH/regular/placebo	3-Month crossover trial with a then continuation of the more successful therapy for 1 year SU lowered HbA_{1c} levels (7.0% vs 7.9%; P<.05) and insulin dose (0.39 vs 0.62 U/kg; P<.05) SU better in ⅔ of patients and remained effective at 1 year if continued
Feinglos et al,[116] 1998	37	30 mo	Insulin (NPH with or without regular) and placebo vs insulin/glipizide	HbA_{1c} levels lower in insulin and glipizide group (9.8% vs 11.4%; P<.008) Insulin dose was lower with glipizide (69 vs 87 U; P<.005)
Robinson et al,[117] 1998	19 + 14	12 wk	Insulin/MET vs insulin/placebo	2 Crossover studies with 19 with 1 g of MET and 14 with 1 to 2.5 g of MET HbA_{1c} and FPG levels improved (1.6%-2.4%; P<.003) Lower triglycerides and LDL levels (P<.032) in MET group
Lopez-Alvarenga et al,[118] 1999	29	3 mo	Placebo vs acarbose vs bedtime NPH	All subjects already taking SUs Acarbose improved FPG levels (P = .05) but not HbA_{1c} levels Bedtime NPH (mean dose 19 U) decreased FPG and HbA_{1c} levels (P<.01)
Fritsche et al,[119] 2000	13	10 wk	MDI/MET vs MDI/placebo	Intensive MDI modeled on the Diabetes Control and Complications Trial Since goals were by SMBG, MET did not lower HbA_{1c} levels but did decrease insulin dose by 30%
Ponssen et al,[120] 2000	31	5 mo	Insulin/MET vs insulin/placebo	Significantly reduced insulin dose (−9 U; P<.001), HbA_{1c} levels (−0.7%; P = .005), and total cholesterol levels (−7 mg/dL; P = .005)

(continued)

eTable 3. Randomized Controlled Trials of Combination Therapy With Available Oral Agents and Human or Animal Insulin (cont)

Source	No. of Patients	Study Length	Treatment Arms	Outcomes (Glucose Reported as mg/dL)
				Parallel Trials
Osei et al,[121] 1984	22	16 wk	Insulin/glyburide vs insulin/placebo	Combination therapy decreased FBG levels (252 vs 286; $P<.05$) and HbA_{1C} levels (9.62% vs 10.92%; $P<.05$) vs placebo and increased C-peptide levels ($P<.05$) vs baseline No difference in results of lipid levels or glucose tolerance tests between groups
Falko and Osei,[122] 1985	22	16 wk	Fixed dose insulin/glyburide or insulin/placebo	FBG ($P<.01$) and HbA_{1C} ($P<.05$) levels decreased with glyburide C-peptide levels were higher in responders than nonresponders (5 vs 4; $P<.01$) No difference in lipid levels between groups
Quatraro et al,[123] 1986	30	1 y	Insulin vs insulin/glicazide	Combined therapy lowered HbA_{1C} levels (8.7% vs 9.0%; $P<.05$), FPG levels (152 vs 165; $P<.05$), and insulin dose (40%) 2 Nonresponders were excluded
Mauerhoff et al,[124] 1986	22	16 wk	Insulin/glibenclamide vs insulin/placebo	Decreased FPG (179 to 147; $P<.02$) and triglyceride ($P<.05$) levels in SU group vs baseline Increased hypoglycemic episodes (107 vs 25) despite an 8% to 10% insulin dose reduction (0.5 to 0.45 U/kg; $P<.02$), weight gain (1.3 kg; $P<.02$), and C-peptide levels ($P<.02$) in SU group
Gutniak et al,[125] 1987	20	325 d	NPH/regular/glyburide vs NPH/regular/placebo	HbA_{1C} levels improved in both groups Decreased insulin dose (63 to 35 U; $P<.001$) for glyburide in insulin and SU group Weight gain was greater with SU use vs control (6.0 kg; $P<.005$ vs baseline, vs 2.9 kg [not significant])
Reich et al,[126] 1987	20	4 mo	Insulin/glyburide vs glyburide/placebo	Patients hospitalized to add SU or placebo with resultant decrease in insulin dose, then HbA_{1C} levels worsened from 9.9% to10.9% in placebo group
Bachmann et al,[127] 1988	68	24 wk	Glibenclamide/insulin vs insulin	Goal PPBG target level <220 attained by 75% in both groups No difference in FBG and HbA_{1C} levels or hypoglycemia between groups Insulin dose decreased with SU (20 vs 35 U)
Casner[128] 1988	64	12 mo	NPH/regular/SU vs NPH/regular/placebo	HbA_{1C} levels lowered with SU at 3 months (11% to 10%; $P<.05$) but increased at 12 months (13%) C-peptide levels increased ($P<.05$) at 3 months then decreased 42% of patients responded to SU Responder insulin dose of 39 U/d vs 79 U/d at a cost increase of 50% for responders No difference in weight gain between groups
Lawrence and Abraira,[129] 1988	20	16 wk	Insulin/glyburide vs insulin/placebo	Glyburide decreased HbA_{1C} levels ($P<.05$), then HbA_{1C} levels increased with placebo (9.9%-10.9%), but remained unchanged (8.9%) in SU group
Lins et al,[130] 1988	20	12 wk	Insulin/glibenclamide vs insulin/placebo	SU improved HbA_{1C} levels from 8.3% to 7.0% and reduced insulin dose by 30%
Klein,[131] 1991	50	12 mo	Glibenclamide/MET vs glibenclamide/insulin	Insulin dose 12 to 40 U No difference in HbA_{1C} or PPG or lipid levels between groups Treatment "failures" (inadequate control) were withdrawn from analysis Insulin type not specified Authors of Scientific Review note that the study design is questionable
Groop and Widen,[132] 1991	36	6 mo	Twice-daily intermediate/regular insulin vs glibenclamide/MET vs 6 wk 3 times/d intermediate/regular insulin, then back to SU	All arms showed decrease in FPG and HbA_{1C} levels by 30% Insulin 3 times/d lowered CBG levels but return to SU alone brought CBG levels back to baseline level Insulin produced a weight gain of 5 kg No change in lipid levels between groups Insulin not specified (authors acknowledge Nordisk)
Yki-Jarvinen et al,[25] 1992	153	3 mo	Morning NPH/SU vs evening NPH/SU vs 2 times/d 70/30 vs MIT vs SU only	Insulin lowered HbA_{1C} levels ($P<.001$) and improved patient well being vs SU ($P<.001$) Less weight gain in evening insulin group only ($P<.05$)
Riddle et al,[134] 1992	21	16 wk	Predinner 70% NPH/30% regular and glyburide vs predinner 70% NPH/30% regular	SU lowered HbA_{1C} levels (−1.3% vs −0.8%; $P<.05$), FBG levels ($P<.05$), despite lower insulin dose (50 U vs 101 U; $P<.01$) Similar weight gain between groups
Chiasson et al,[135] 1994	354	1 y	Acarbose and either diet, MET, SU, or insulin	HbA_{1C} levels improved compared with placebo in all groups (all $P<.01$) No change in FBG or lipid levels among groups
Shank et al,[136] 1995	30	1 y	1. SU; 2. SU vs bedtime NPH+SU vs bedtime NPH; 3. and 4. titrated NPH	4-Phase study FPG and HbA_{1C} levels best controlled with bedtime NPH and SU (all $P<.05$) Weight gain correlated with decreased glycosuria (vs SU) levels Lipid levels improved significantly with insulin vs SU and as glucose control improved

(continued)

eTable 3. Randomized Controlled Trials of Combination Therapy With Available Oral Agents and Human or Animal Insulin (cont)

Source	No. of Patients	Study Length	Treatment Arms	Outcomes (Glucose Reported as mg/dL)
			Parallel Trials (cont)	
Clauson et al,[137] 1996	39	1 y	Bedtime NPH/ glibenclamide vs MDI	Nonobese patients (BMI approximately 25.6 kg/m^2) HbA_{1C} levels better at 6 months in MIT (6.8% vs 8.2%; $P<.001$) but not at 12 months (7.5% vs 7.8%; not significant) More weight gain with MIT (5.6 vs 3.3 kg; $P = .06$) No change in lipid levels in both groups
Chow et al,[97] 1995	53	6 mo	Bedtime NPH/SU or bedtime NPH/MET vs twice-daily 70% N/30% regular	Control improved in both groups, no difference in FBG or HbA_{1C} levels Combined therapy had decrease in insulin dose (15 vs 57 U; $P<.0001$) and weight gain ($P<.005$) Triglyceride levels lower ($P<.02$) and well being and quality of life better with insulin only ($P<.05$)
Wolfenbuttel et al,[138] 1996	95	6 mo	Twice-daily 70% NPH/30% regular vs bedtime NPH/glibenclamide vs twice-daily NPH/ glibenclamide	HbA_{1C} levels improved in all groups HbA_{1C} levels <8% in 8 insulin and SU patients; 11 taking bedtime NPH and SU; 15 taking twice-daily 70 NPH/30 regular Mean NPH dose of 34 U in 70 NPH/30 regular group vs 23 U for once-daily NPH group Significantly more patients had HbA_{1C} levels >9% while taking morning NPH and SU No difference in weight gain or hypoglycemia between groups
Colwell,[139] 1996	153	27 mo	Once- or twice-daily insulin vs intensive treatment: (1) bedtime insulin; (2) add daytime glipizide; (3) twice-daily insulin no SU; (4) MDI	In intensive group (36% of patients taking MDI), HbA_{1C} levels decreased 2.1% with mean insulin dose of 100 U Near maximal HbA_{1C} levels decrease occurred at bedtime insulin and glipizide stage
Abraira et al,[6] 1998	153	27 mo	Bedtime insulin vs bedtime insulin/morning glipizide vs twice-daily insulin vs MDI	4-Phase trial with intensive patients' goal HbA_{1C} levels <7.3% HbA_{1C} levels for bedtime insulin of −1.4%, bedtime insulin and glipizide of −1.9%, twice-daily no better, and MDI of −2.4% MDI significantly increased hypoglycemic events and insulin dose
Relimpio et al,[141] 1998	47	4 mo	Insulin vs insulin/MET	Maximum dose MET or 20% increase from baseline insulin dose Weight gain with insulin vs insulin and MET (1.2 vs 0.3 kg) Significantly better HbA_{1C} levels (−1.9% vs −0.03%; $P<.01$) and total cholesterol and LDL levels ($P<.01$)
Niazi and Muzaffar,[155] 1998	36	20 wk	Insulin vs MET/ glibenclamide	20 to 40 U of bedtime NPH vs MET and glibenclamide Similar reduction in BG levels between groups 50% of insulin group (n = 9) dropped out
Kelley et al,[142] 1998	145	24 wk	Insulin/acarbose vs insulin/placebo	HbA_{1C} levels reduced from approximately 8.7% by 0.58% ($P<.001$) more than placebo Despite more gastrointestinal tract adverse effects (acarbose), no difference in drop-out rates between groups
Riddle and Schneider,[143] 1998	145	24 wk	Supper premix 70% NPH/30% regular/glimepiride vs premix 70% NPH/30% regular/placebo	Treated to target FPG level <140 HbA_{1C} (7.7%) and FPG levels (137-138) equivalent at end of study Lower insulin dose in SU group (49 vs 78 U/d; $P<.001$) No differences in hypoglycemia, weight gain, or lipid levels between groups
UKPDS 33 et al,[6] 1998	3867	10 y	Intensive (SU or insulin) vs diet	Intensive (insulin or SU) decreased HbA_{1C} levels (7.0% vs 7.9%) and complications (12%), and increased frequency of severe hypoglycemic episodes by 3% and weight gain (insulin, 4.0 kg vs SUs, <2.6 kg)
Yki-Jarvinen et al,[144] 1999	96	1 y	Bedtime NPH and either SU and placebo; MET and placebo; SU and MET; or morning NPH	Less weight gain in NPH and MET (0.9 kg; $P<.001$ vs 3.6-4.6 kg for all other groups) Lower HbA_{1C} levels in NPH and MET ($P<.05$) and fewer episodes of hypoglycemia ($P<.05$)
Aviles-Santa et al,[145] 1999	43	24 wk	Insulin/MET vs insulin/ placebo	MET lowered HbA_{1C} levels (2.5% vs 1.6%; $P = 0.04$) and insulin dose (29%; $P<.002$); and less weight gain (0.5 vs 3.2 kg; $P = .07$)
Bastyr et al,[146] 1999	423	2 mo	Prandial lispro/SU vs SU/bedtime NPH vs prandial lispro/bedtime NPH	HbA_{1C} levels lower with lispro and SU vs NPH and SU (−1.21 vs −1.40; $P = .003$) and both lower than baseline ($P<.001$) FBG levels lowest for NPH and SU ($P<.001$) Lowest frequency of nocturnal hypoglycemic episodes in lispro and SU ($P = .004$)
Turner,[94] 1999	4075	9 y	Diet vs insulin vs SU vs MET	HbA_{1C} levels <7%: diet group, 9%; insulin group, 28%; SU group, 24%; MET group, 18% FPG levels <140: diet group, 8%; insulin group, 42%; SU group, 24%; MET group, 13% MET patients were obese Progressive need for multiple therapies: 50% at 3 years; 75% at 9 years

Abbreviations: BG, blood glucose; FBG, fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDI, multiple daily injections; MET, metformin; NPH, isophane insulin (neutral protamine Hagedorn); PP, postprandial; SMBG, self-monitored blood glucose; SU, sulfonylurea.

SI conversion factors: for glucose, to convert mg/dL to mmol/L, multiply by 0.0555; for cholesterol, to convert mg/mL to mmol/L, multiply by 0.0259.

*Ultralente is insulin zinc extended.

†Authors of Scientific Review note that troglitazone data have not been included since it has been taken off the US market. Many trials with oral insulin do not specify insulin regimens or concomitant insulin adjustment protocols during the trial making it difficult to interpret reported outcomes.

EXHIBIT E

Long-Term Exposure of Rat Pancreatic Islets to Fatty Acids Inhibits Glucose-induced Insulin Secretion and Biosynthesis through a Glucose Fatty Acid Cycle

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Abstract

We tested effects of long-term exposure of pancreatic islets to free fatty acids (FFA) in vitro on B cell function. Islets isolated from male Sprague-Dawley rats were exposed to palmitate (0.125 or 0.25 mM), oleate (0.125 mM), or octanoate (2.0 mM) during culture. Insulin responses were subsequently tested in the absence of FFA. After a 48-h exposure to FFA, insulin secretion during basal glucose (3.3 mM) was several-fold increased. However, during stimulation with 27 mM glucose, secretion was inhibited by 30–50% and proinsulin biosynthesis by 30–40%. Total protein synthesis was similarly affected. Conversely, previous palmitate did not impair α-ketoisocaproic acid (5 mM)-induced insulin release. Induction and reversibility of the inhibitory effect on glucose-induced insulin secretion required between 6 and 24 h. Addition of the carnitine palmitoyltransferase I inhibitor etomoxir (1 μM) partially reversed (by > 50%) FFA-associated decrease in secretory as well as proinsulin biosynthetic responses to 27 mM glucose. The inhibitory effect of previous palmitate was similar when co-culture was performed with 5.5, 11, or 27 mM glucose. Exposure to palmitate or oleate reduced the production of $^{14}CO_2$ from D-[U-^{14}C]glucose, and of $^{14}CO_2$ from D-[3,4-^{14}C]-glucose, both effects being reversed by etomoxir. Conclusions: long-term exposure to FFA inhibits glucose-induced insulin secretion and biosynthesis probably through a glucose fatty acid cycle. (*J. Clin. Invest.* 1994. 93:870–876.) Key words: B cell insensitivity • fatty acids • insulin secretion • non-insulin-dependent diabetes mellitus • pancreatic islet

Introduction

The importance of FFA in the regulation of insulin secretion has not been thoroughly assessed. During nonstimulatory conditions, endogenous triacylglycerols are important fuels for intermediary metabolism of pancreatic islets (1). However, effects on insulin secretion are less impressive. Although FFA have been shown in vivo and in vitro to stimulate insulin secretion (2, 3), such stimulation is usually moderate, suggesting that FFA do not play a major role in regulation of insulin secretion.

Theoretically, elevated FFA, such as occur during starvation and diabetes, could influence insulin secretion in a major way if a glucose–fatty acid cycle were operative in the B cell. The concept of such a cycle was proposed by Randle et al. (4) many years ago. It encompasses the notion that the relationship between glucose and fatty acid breakdown is reciprocal and not dependent. Accordingly, increased oxidation of fatty acids, such as occurs in fasting and diabetes, will inhibit the metabolism of glucose (by several mechanisms, see reference 5 for review). This relationship between fatty acid oxidation and glucose metabolism has been documented in tissues such as heart muscle and liver (5). However, the existence of a glucose fatty acid cycle has not been demonstrated in pancreatic islets. Rather, most previous studies have seemed to disprove its existence. Hence in short-term experiments fatty acid oxidation did not negatively affect glucose oxidation (6). Furthermore, in a detailed study on the effects of a carnitine palmitoyl transferase I(CPT I)[1] inhibitor, methyl-palmoxirate, this inhibitor of long-chain fatty acid oxidation failed to affect D-[U-^{14}C]-glucose oxidation, and did not enhance but, instead, diminished glucose-induced insulin release (7). More recently the CPT I inhibitor 2-bromopalmitate was shown to enhance glucose-induced insulin secretion in a clonal β cell line of HIT cells (8). However, it is difficult to decide whether these results in insulinoma cells are applicable to normal B cells.

Previous studies utilized mostly short-term protocols to study the interactions between FFA and glucose. It seemed possible that long-term exposure to FFA could induce effects different from those exerted by short-term exposure. This hypothesis was tested by infusing a lipid solution, Intralipid, for varying durations into rats (9). Glucose-induced insulin secretion was measured subsequent to infusion using the perfused pancreas. The effects of Intralipid turned out to be biphasic in time. After 3 h of infusion, glucose-induced insulin secretion was enhanced whereas it was decreased after 24 h and even more so after 48 h of infusion. These results indicated that the long-term hyperlipidemia inhibited glucose-induced insulin secretion.

Hyperlipidemia induces insulin resistence (10, 11) as well as many changes in nutrient and hormonal levels, all of which could potentially affect B cell function. Although the infusion experiments provided some data suggesting that the effects observed were related to fatty acid oxidation (9), they did not provide definite evidence that the effects of hyperlipidemia were directly caused by elevated FFA. Furthermore, even if such were the case, the relevance to physiology would be in doubt since Intralipid contains a much higher proportion of unsaturated fatty acids than plasma, its major fatty acid component being linoleic acid (9), whereas the dominating FFA in plasma are oleic and palmitic acid. The first aim of the present study was therefore to directly test for time-dependent effects of different concentrations of palmitate and of oleate on glucose-

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1. *Abbreviations used in this paper:* CPT I, carnitine palmitoyl transferase I; PDH, pyruvate dehydrogenase.

induced insulin secretion. The second aim was to test for effects of FFA on the other major effect of glucose on the B cell, namely, stimulation of insulin biosynthesis. The third aim was to test for coupling of FFA effects on secretion with effects on islet metabolism.

Methods

Materials. Palmitate, oleate, and octanoate (sodium salts) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium 2-[b-(4-chlorophenoxy)-hexyl]oxirane-2-carboxylate (B-827-33, etomoxir) was from ASAT AG (Zug, Switzerland). L-[4,5-^{3}H]leucine (sp act 62 Ci/mmol) was from Amersham International (Amersham, Bucks, UK). Undiluted guinea pig anti-porcine insulin serum was from ICN ImmunoBiologicals (Lisle, IL). Protein A-Sepharose CL-4B was from Pharmacia (Uppsala, Sweden). D-[U-^{14}C]glucose (sp act 320 mCi/mmol) and D-[3,4-^{14}C]glucose (53.6 mCi/mmol) were from New England Nuclear (Boston, MA). Hyamine hydroxide was from Packard Instrument Co. (Downers Grove, IL).

Animals. Male Sprague-Dawley rats were obtained from B & K Universal, Stockholm, Sweden. They had free access to water and a standard pelleted diet (EWOS-ALAB Brook Stock Feed for rats and mice). The pellets contained on a weight basis 51.5% carbohydrate, 22% protein, and 5% fat. They were exposed to a 12-h/12-h light (0600–1800)/dark cycle. At the time of experiments they weighed between 200 and 300 g. The experimental protocols were approved by the Stockholm Ethical Committee for Research on Animals.

Isolation and culture of islets. Animals were killed by decapitation and pancreatic islets of Langerhans isolated as previously described (12), using digestion with collagenase obtained from *Clostridium histolyticum* (Boehringer Mannheim Gmb H, Mannheim, FRG). Digestion and sedimentation of islets were carried out in Hanks' solution containing 5.5 mM glucose (Statens Bakteriologiska Laboratorium, Stockholm). Islets were then handpicked under a stereomicroscope and transferred to Petri dishes (Steriline, Teddington, UK) containing culture medium RPMI 1640 (Flow Laboratories, Erwing, UK) supplemented with 100 U/ml benzylpenicillin, 0.1 mg/ml streptomycin, 2 mM L-glutamine, and 10% (vol/vol) heat-inactivated fetal calf serum (Sigma Chemical Co.). The concentration of glucose in culture media was 11.0 mM when not otherwise indicated. Islets were cultured free-floating at 37°C, with an atmosphere of 5% CO_2/95% air. The time period of culture was 48 h except in time course studies of induction and reversibility.

Table I. Effect of Ethanol and Etomoxir on Glucose-induced Insulin Release and Insulin Content of Rat Islets Maintained in Tissue Culture

Culture conditions	No. of experiments	Insulin release in final incubations, Glucose (mM) 3.3	27	Insulin content
		ng/islet · 60 min		*ng/islet*
No additions	5	0.96±0.1	29±1.4	26±1.2
1% ethanol	5	0.88±0.12	27±1.7	28±1.9
2% ethanol	5	0.88±0.08	29±1.6	26±1.8
2% ethanol + 1 μM etomoxir	5	0.92±0.16	26±1.9	—

Islets were cultured for 48 h in RPMI 1640 with 11 mM glucose, containing ethanol and etomoxir at the concentrations listed; insulin release was determined in 60-min final incubations. No significant differences were found between experimental conditions.

Fatty acids (sodium salts) were dissolved in 95% ethanol before being added to culture media. The final concentration of ethanol in medium was 1% or 2% (vol/vol) at the concentrations of FFA used in this study. Neither of the concentrations used during culture affected the subsequently tested insulin release nor islet insulin content (Table I). Control conditions with 1% or 2% ethanol during culture were included in each experiment.

Batch-type incubations of islets. After 48 h of culture in RPMI 1640, islets were always preincubated for 30 min in KRB medium (13) with the following composition: Na^+ 143.5 mM, K^+ 5.8 mM, Ca^{2+} 2.5 mM, Mg^{2+} 1.2 mM, Cl^- 124.1 mM, PO_4^{3-} 1.2 mM, SO_4^{2+} 1.2 mM, CO_3^{2-} 25 mM, pH 7.4, and supplemented with 10 mM Hepes, 2 g/liter BSA (fraction IV, Sigma Chemical Co.), and 3.3 mM glucose. Except for variations in glucose, this buffer was used throughout the study.

For insulin secretion studies, islets were selected after preincubation in batches of three islets in 200 μl of KRB containing either 3.3 or 27 mM glucose. Islets were incubated at 37°C for 60 min. Triplicates or quadruplicates were run for each experimental condition. Incubations were carried out in a water bath with continuous shaking and in an atmosphere of 95% O_2/5% CO_2. At the end of incubations, aliquots of the incubation media were removed for insulin assay. Islets which, in final incubations, were exposed to 3.3 mM glucose were retrieved for later determination of islet insulin content.

Proinsulin and total protein biosynthesis. For the study of proinsulin-insulin and total protein biosynthesis, groups of 10 islets were incubated as duplicates in polystyrene RIA tubes containing 100 μl of KRB medium supplemented with 50 μCi/ml L-[4,5-^{3}H]-leucine and 3.3 or 27 mM glucose at pH 7.4 for 2 h at 37°C (without shaking) in 95% O_2/5% CO_2. After incubations, the medium containing radioactive leucine were carefully removed by suction. The islets were then washed twice with Hanks' solution containing nonradioactive leucine (10 mM). The washed islets were sonicated in 200 μl of redistilled water (10 × 2 s., model B-12 sonifier, setting 4; Branson Ultrasonics Corp., Danbury, CT). The sonicates were then centrifuged (10,000 *g*, 10 min) at 4°C and the supernatants were used for subsequent analysis.

Proinsulin-insulin biosynthesis was measured by immunoprecipitation with undiluted antiinsulin serum (in excessive amount), and separation of immune complexes with protein A-Sepharose CL-4B as described (14). Total protein synthesis was determined by measurements of the radioactivity in the 10% TCA-precipitable fraction of the homogenate.

Glucose oxidation. The production of $^{14}CO_2$ from D-[U-^{14}C]-glucose was measured basically as previously described (15). Briefly, duplicate groups of 10 islets each were placed in wells (central well, Kontes Co., Vineland, NJ) containing 100 μl of KRB medium supplemented with trace amounts of D-[U-^{14}C]glucose plus nonradioactive glucose to a final concentration of either 3.3 or 27 mM. The wells were suspended in 20-ml scintillation vials, which were gassed with O_2/CO_2 (95:5) and capped airtight with rubber membranes. The vials were shaken continuously for 90 min at 37°C in a water bath. Islet metabolism was stopped by an injection of 100 μl of 0.2 M HCl into the wells. This was immediately followed by injection of 250 μl of hyamine hydroxide into the outer vial. After a further incubation for 2 h at 37°C to allow the liberated CO_2 to be trapped by the hyamine, 5 ml of scintillation fluid was added to each vial and the radioactivity was counted in a Packard Instruments Tricarb model 1900 TR scintillation analyzer. Blank incubations were treated identically. Oxidation of glucose was calculated as picomoles of glucose converted to [^{14}C]CO_2 per 10 islets/90 min.

The procedure used to measure the generation of $^{14}CO_2$ from D-[3,4-^{14}C]glucose was identical to that for D-[U-^{14}C]glucose oxidation described above except for using 50 μl of KRB, which contained 0.4–1.0 μCi/ml D-[3,4-^{14}C]glucose, and 120 min for incubation as previously reported (16).

Measurements of insulin release and islet insulin content. Insulin was measured by RIA using rat insulin as standard, monoiodinated porcine insulin as tracer, and charcoal premixed with Dextran T 70 (Pharmacia, Uppsala) to separate antibody bound insulin from free

insulin (17). Insulin antibodies had been raised in our laboratory against porcine insulin.

For the determination of islet insulin contents, three islets each were put into 200 μl of acid-ethanol (0.18 M HCl in 95% ethanol); insulin was extracted overnight at 4°C after sonification as previously described (18).

Presentation of results. All results are expressed as the mean±SE. Significance testing was performed using two-tailed Student's *t* test for unpaired data and one-way ANOVA with the Student-Newman-Keuls' test.

Results

Effects of a 48-h exposure to FFA on glucose-induced insulin secretion and insulin content. The effects of 48 h of culture with palmitate, oleate, or octanoate are shown in Table II. Previous exposure to all three fatty acids increased fourfold or more insulin release during subsequent incubations with 3.3 mM glucose, i.e., a nonstimulating concentration of the hexose. Contrariwise, all these three fatty acids inhibited insulin release during stimulation with 27 mM glucose. With palmitate, inhibition was more marked after 0.25 mM than after 0.125 mM of this fatty acid. At equimolar concentrations of 0.125 mM, the inhibitory effect after previous palmitate was 30%, and after previous oleate 42%. The incremental response to 27 mM glucose was inhibited by 82% after palmitate and by 86% after oleate. All three fatty acids reduced islet insulin contents (Table II). At equal concentrations (0.125 mM), the reduction was 23% after palmitate and 40% after oleate.

Table II. Effects of 48 h of Exposure to Fatty Acids with or without Etomoxir on Insulin Release and Islet Insulin Content

Culture conditions	No. of experiments	Insulin release in final incubations, Glucose (mM) 3.3	27	Insulin content
		ng/islet · 60 min		*ng/islet*
Control (1% ethanol +1 μM etomoxir)	5	1.1±0.1	36±1.7	28±1.8
0.25 mM palmitate	5	5.0±0.5*	19±1.5*	16±1.0*
0.25 mM palmitate +1 μM etomoxir	5	4.4±0.7*	26±1.6*‡	19±1.7§
0.125 mM oleate	5	4.7±0.8*	21±1.6§	17±1.5*
0.125 mM oleate +1 μM etomoxir	5	3.5±0.6*	29±2.0§‖	22±1.7‡
Control (1% ethanol +1 μM etomoxir	3	1.3±0.1	33±2.0	27±2.4
0.125 mM palmitate	3	5.2±0.9*	24±1.5*	21±1.4¶
0.125 mM palmitate +1 μM etomoxir	3	4.7±0.7*	31±1.9‖	25±1.3‡
Control (2% ethanol)	5	1.7±0.2	33±1.4	—
Octanoate 2.0 mM	5	6.1±0.6*	13±1.5*	22±1.8¶
Octanoate 2.0 mM +1 μM etomoxir	5	4.3±0.5*	15±1.1*	21±4.1¶

Insulin release was measured in 60-min final incubations after 48 h of culture followed by a 30-min preincubation in KRB containing 3.3 mM glucose. Data are means±SE. ¶ $P < 0.05$; § $P < 0.01$; and * $P < 0.001$ compared with control. ‡ $P < 0.05$; ‖ $P < 0.01$ for the effect of etomoxir.

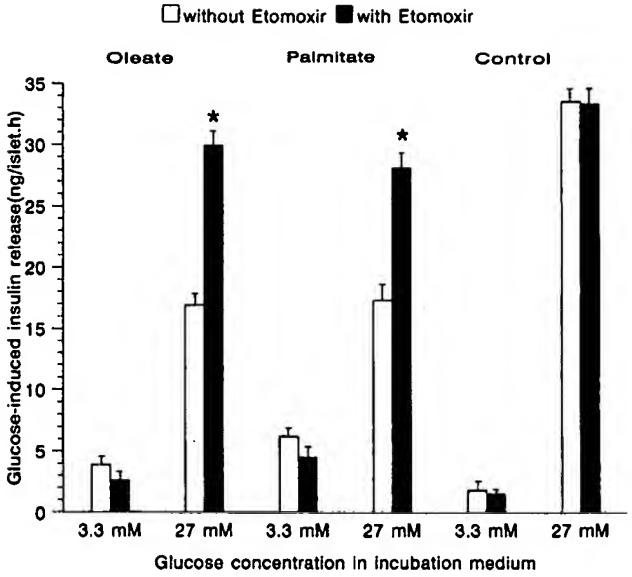

Figure 1. Effects of etomoxir (1 μM) present after culture on FFA-associated impairment of insulin response to glucose. Islets cultured with palmitate or oleate (but not etomoxir) for 48 h were transferred to the KRB medium with or without 1 μM etomoxir, and underwent preincubation (30 min) and final incubation (60 min) to measure glucose-induced insulin release. Data are means±SE. of five or six experiments. * $P < 0.01$ for effect of etomoxir. Control means islets cultured without palmitate.

Influence of etomoxir on FFA-induced changes of insulin secretion. Etomoxir, one of the mitochondrial CPT I inhibitors containing oxirane carboxylates (19, 20), was used to test whether inhibition of the β-oxidation of long-chain fatty acids, such as palmitate and oleate, could reverse their effects on insulin secretion (Table II). Addition of 1 μM etomoxir to culture media did not per se influence basal or glucose-induced insulin secretion (Table I). Addition of etomoxir to culture media containing fatty acids did not significantly affect the fatty acid-stimulated insulin secretion in the presence of 3.3 mM glucose. Etomoxir during culture with palmitate or oleate partially (by 75% for 0.125 mM palmitate, and by 48% for 0.125 mM oleate) reversed the inhibitory effects of these fatty acids on insulin response to 27 mM glucose.

Specificity of the etomoxir effect was tested by adding the CPT I inhibitor to culture media containing octanoate. The oxidation of this fatty acid is known not to involve the CPT enzyme (21). In our experiments, previous etomoxir together with octanoate failed to significantly affect the inhibitory effect on glucose-induced insulin secretion exerted by culture with this fatty acid (Table II).

Etomoxir partially reversed the reduction in islet insulin content brought about by palmitate or oleate. However, the reduction in islet insulin content brought about by octanoate was not affected.

In another series of experiments, etomoxir was added from preincubation and onward, but not during culture. Also in these experiments, etomoxir partially reversed FFA-associated impairment of 27 mM glucose-induced insulin secretion (Fig. 1). Finally, exposure to etomoxir was tested from the beginning of the culture period up to the end of final incubations (five experiments). Also, this protocol resulted in near total reversibility by etomoxir. Thus secretion was 17±1.2 ng/islet · h in the absence and 24±1.1 ng/islet · h in the presence of

Table III. Time Course of Induction of Palmitate-induced Effects on Insulin Release and Insulin Content

	Insulin release in final incubations					
	Control (1% eth)		Previous palmitate			
	Glucose (mM)		Glucose (mM)		Insulin content	
Duration of culture	3.3	27	3.3	27	Control	Previous palmitate
h	*ng/islet · 60 min*				*ng/islet*	
6	1.2±0.1	40±1.5	2.5±0.3*	38±2.1	29±3.2	19±3.3*
24	1.3±0.1	35±1.4‡	3.1±0.4*	29±1.7§	23±3.9	16±2.9§
48	1.3±0.1	28±2.5‖	2.1±0.2*	14±1.2*	23±3.1	15±2.9§

Islets were cultured with or without 0.125 mM palmitate for 6, 24, and 48 h. Results are means±SE of five experiments. § $P < 0.01$; * $P < 0.001$ when compared with control (1% ethanol). ‡ $P < 0.05$, ‖ $P < 0.01$ when compared with 6-h exposure group.

etomoxir for culture with 0.25 mM palmitate; and 19±1.0 ng/islet · h in the absence and 26±1.5 ng/islet · h in the presence of etomoxir for culture with 0.125 mM oleate, the normal control response being 26±1.9 ng/islet · h.

Time course for induction and reversibility of palmitate-induced effects. Induction time for FFA effects on insulin secretion was studied by exposing islets to 0.125 mM palmitate for 6, 24, or 48 h. As can be seen from Table III, the basal insulin secretion at 3.3 mM glucose was elevated near-maximally already after 6 h of palmitate exposure when compared with control conditions (culture at RPMI 1640 with 1% ethanol). In contrast, the insulin response to 27 mM glucose was significantly reduced only after 24 h exposure. A 48-h exposure was needed to induce a marked effect (lowering to 50% of the control).

Insulin contents in palmitate-exposed islets were reduced already after a 6-h exposure (to 60% of the control). With longer exposure, the absolute values decreased further; however, due to some reduction with time of culture in insulin contents of control islets, there was no further drop in the ratio of insulin content between palmitate-exposed and control islets.

The same experiments which were used to evaluate induction were continued to assess also the time of reversibility. Islets which had been exposed to palmitate or ethanol for 48 h (and not used for measurements of induction) were transferred to standard RPMI 1640 medium with 11.0 mM glucose and 2 mM L-glutamine but without palmitate. After 6 h of recovery culture, the influence of palmitate exposure persisted both with regard to the increased basal and decreased stimulated insulin secretion (Fig. 2). However, after 24 h, both basal and stimulated insulin secretion were restored to normal. Insulin contents in these islets also increased significantly (from 29±2.7 ng/islet at 6 h, to 38±55.4 μU/islet at 24 h); however, these contents were still lower than those of control condition (47±2.2 ng/islet, $P < 0.01$).

Effect of glucose concentration during culture on effects of palmitate. To evaluate the possible interaction between hyperglycemia and FFA on glucose-induced insulin release, the exposure of islets to 0.125 mM palmitate were compared for three different glucose concentrations (5.5, 11.0, and 27 mM) during culture. Increasing the glucose concentration from 5.5 to 11 mM did not affect basal release at 3.3 mM glucose in control culture. However, the increase in glucose during culture augmented the stimulatory effect of palmitate at 3.3 mM glucose (Table IV). Increasing the glucose concentration to 27 mM led to 5.7-fold higher release at 3.3 mM glucose. Co-culture of palmitate with 27 mM glucose did not further increase subsequent release at 3.3 mM glucose. Hence culture at an intermediate glucose concentration enhanced, whereas culture at a high glucose concentration diminished the stimulatory effect of previous palmitate per se.

The inhibitory influence of palmitate, on the other hand, was not affected by increasing the glucose concentration during culture. Thus no difference was apparent in FFA-induced attenuation of 27 mM glucose-induced insulin release whether the culture medium contained 5.5, 11, or 27 mM glucose (Table IV). Also the magnitude of palmitate-induced decrease in insulin contents seemed unaffected by glucose concentration during culture, the decrease being additive to that brought about by culture with 27 mM glucose alone.

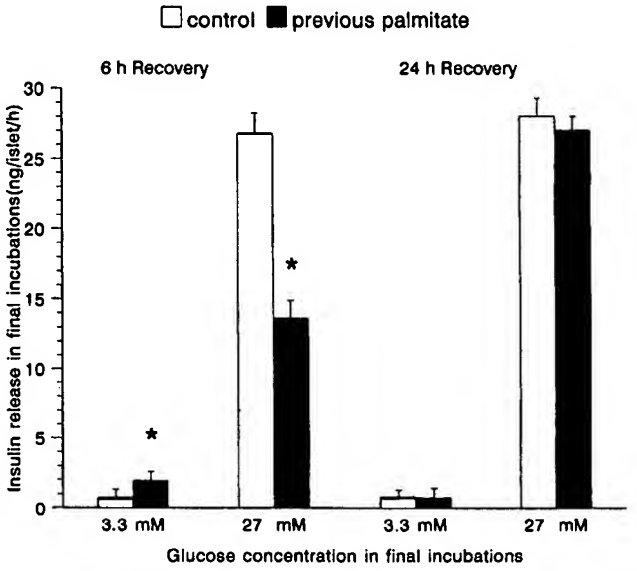

Figure 2. Reversibility of palmitate-induced impairment of insulin response to glucose in cultured rat islets. After 48 h of culture with 0.125 mM palmitate, islets were transferred to standard RPMI 1640 medium (with 11 mM glucose and 2 mM glutamine) for an additional 6- or 24-h of recovery culture. Insulin release was subsequently measured in batch-type incubations in KRB with 3.3 or 27 mM glucose. Results are means±SE. of five experiments. * $P < 0.001$ when compared with control.

Table IV. Effects of Glucose Concentration during Culture on Palmitate-induced Effects on Insulin Release and Insulin Contents

Glucose in culture medium	Insulin release in final incubations				Insulin content	
	Control		Previous palmitate			
	Glucose (mM)		Glucose (mM)			
	3.3	27	3.3	27	Control	Previous palmitate
mM	*ng/islets · 60 min*				*ng/islet*	
5.5	1.1±0.1	23±0.8	3.9±0.5*	18±1.6‡	31±1.9	21±3.3‡
11.0	1.0±0.1	24±0.6	6.5±0.3*	20±1.0‡	37±2.5	18±1.3*
27.0	6.4±0.5	26±0.5	7.0±0.4	21±0.8*	25±2.5	16±2.9‡

Islets were cultured in RPMI 1640 medium containing the three different concentrations of glucose, in the presence or absence of 0.125 mM palmitate for 48 h. Insulin responses to 3.3 or 27 mM glucose were measured in the 60-min final batch-type incubations. Data are means±SE of four experiments. * $P < 0.001$ and ‡ $P < 0.05$ when compared with control.

Effect of palmitate on α-ketoisocaproic acid-induced insulin release. To examine the specificity of inhibition by fatty acids of glucose-induced insulin release, we tested the effects of palmitate during culture on subsequent release induced by 5 mM α-ketoisocaproic acid. In contrast to its inhibitory effect on glucose-induced insulin release, previous 0.125 mM palmitate failed to inhibit and even slightly increased α-ketoisocaproic acid-induced insulin secretion. Thus release after previous palmitate was 16±1.2 vs. 13±0.75 ng/islet · 60 min during control conditions, $P < 0.05$).

Effects of FFA on proinsulin and total protein biosynthesis in islets. Effects of 48 h of exposure to 0.125 mM palmitate or oleate on islet biosynthesis are shown in Table V. At 3.3 mM glucose neither palmitate nor oleate influenced incorporation of [^{3}H]leucine into proinsulin-insulin. However, incorporation at 27 mM glucose was inhibited by both fatty acids. Inhibition of the absolute rate of biosynthesis amounted to 40% for palmitate and 28% for oleate. Also the effects of glucose on total protein synthesis in the presence of 27 mM glucose were inhibited by palmitate and oleate. The inhibitory effects on total protein synthesis were no less than on proinsulin biosynthesis. Hence the fatty acids did not change the relative contribution of proinsulin synthesis to total protein synthesis.

Addition of 1 μM etomoxir to culture media significantly reversed the inhibitory effects of palmitate and oleate on glucose-stimulated proinsulin biosynthesis. There was a tendency, albeit nonsignificant, for reversal of inhibition of total protein synthesis (Table V).

Effects of FFA on glucose oxidation. The rate of D-[U-^{14}C]-glucose oxidation at 3.3 mM of the hexose was not influenced by culture with palmitate or oleate (Table VI). However, oxidation in the presence of 27 mM glucose was inhibited by 24% and 32% in islets cultured in medium containing respectively palmitate or oleate. The co-presence of 1 μM Etomoxir with palmitate or oleate in culture media restored the impaired oxidation rate to normal or near normal.

The rate of D-[3,4-^{14}C]glucose was also studied in order to assess an influence of FFA on the decarboxylation of pyruvate. The production of $^{14}CO_2$ was decreased by culture with palmitate or oleate. The inhibitory effect amounted to 35% after palmitate and 39% after oleate. This inhibition was fully reversible by etomoxir.

Discussion

Our results show that exposure to fatty acids in vitro leads to profound inhibition of glucose-induced insulin secretion. To our knowledge, such an effect has not previously been documented in vitro. The absence of an inhibitory effect in other studies is explainable by the fact that short-term effects were

Table V. Effects of FFA and Etomoxir on Proinsulin and Total Protein Biosynthesis

Culture conditions	Proinsulin synthesis (PI)		Total protein synthesis (TP)		Percent PI of TP	
	Glucose (mM)		Glucose (mM)		Glucose (mM)	
	3.3	27	3.3	27	3.3	27
	10^3 dpm/islet · 2 h				%	
No addition	3.38±0.4	6.45±0.51	9.26±1.02	16.5±1.72	33.4±2.0	39.8±2.0
1% ethanol	2.54±0.26	5.71±0.31	8.74±0.94	17.6±0.73	29.3±1.6	32.6±1.7
1% ethanol + 1 μM etomoxir	2.52±0.27	4.98±0.40	8.15±0.63	14.2±1.25	30.6±1.1	34.9±0.7
Palmitate (0.125 mM)	2.41±0.21	3.41±0.34*	7.98±0.67	9.77±0.71*	30.0±1.0	34.7±2.3
Palmitate + etomoxir	2.70±0.19	4.99±0.65‡	7.73±0.56	13.8±1.97	35.5±2.1	36.0±1.9
Oleate (0.125 mM)	3.14±0.11	4.16±0.19*	9.24±0.71	12.0±0.78*	35.1±1.9	35.2±2.2
Oleate + etomoxir	3.44±0.25	5.46±0.47‡	9.62±0.54	14.8±1.40	36.1±1.7	36.2±0.7

Islets were exposed to fatty acids in culture for 48 h, in the presence or absence of 1 μM etomoxir. Afterward, proinsulin biosynthesis was determined by measuring the incorporation of L-[3,4-^{3}H]leucine, and the total protein synthesis was determined by counting the 10% TCA-precipitable fraction of radioactivity. Values are means±SE of four experiments. * $P < 0.01$ vs. 1% ethanol; ‡ $P < 0.05$ vs. those with FFA only.

Table VI. Effects of 48 h of Exposure to Fatty Acids with or without Etomoxir on Glucose Oxidation in Rat Islets

	Glucose oxidation			
	D-[U-^{14}C]glucose (n = 5)		D-[3,4-^{14}C]glucose (n = 4)	
	Glucose in incubation medium (mM)			
Culture conditions	3.3	27	3.3	27
	pmol/10 islets · 90 min			
No additions (RPMI + 11 mM glucose)	159±20.4	828±78.9	—	—
1% ethanol + 1 μM etomoxir	158±10.7	866±68.4	92±12	391±29
Palmitate 0.125 mM	173±13.4	660±54.1*	102±4.9	260±15‡
Palmitate 0.125 mM + etomoxir	161±11.3	890±88.5§	109±14	383±32‖
Oleate 0.125 mM	151±13.5	592±53.4‡	98±9.2	239±33‡
Oleate 0.125 mM + etomoxir	156±15.3	807±67.1§	98±12	358±34‖

Data are means±SEM of four separate experiments. * $P < 0.05$, ‡ $P < 0.01$ when compared with control (1% ethanol plus 1 μM etomoxir); § $P < 0.05$, ‖ $P < 0.01$ when compared with those exposed to FFA only.

usually investigated (2, 3, 22, 23). Our study shows that induction of inhibition by FFA requires a long induction time, i.e., between 6 and 24 h.

Several observations indicate that the inhibitory effect of long-term FFA is not due to unspecific damage of B cell function. First, the effect was reversible upon removal of FFA on a time scale comparable with that for induction. Second, responses to another nutrient secretagogue, α-ketoisocaproic acid, were not inhibited. Third, an effect of FFA on glucose-induced insulin release was rapidly reversible by etomoxir (see further below).

Our findings strongly indicate coupling between fatty acid oxidation and the time-dependent inhibition of glucose-induced insulin secretion. Etomoxir, which is a CPT I inhibitor (19, 20), thus partially reversed the inhibitory effect of palmitate or oleate. The specificity of the etomoxir effect is highlighted by failure of the drug to significantly reverse the inhibition of glucose-induced insulin secretion caused by previous octanoate. By virtue of being a medium chain fatty acid, octanoate is known to enter mitochondria and to be metabolized without involvement of the CPT I enzyme (21).

We observed that etomoxir can reverse inhibition of glucose-induced insulin secretion not only when added during culture but also when added solely after culture in the absence of exogenous FFA. The efficiency of etomoxir when present solely after culture indicates the importance of breakdown of islet endogenous triacylglycerols for the inhibitory effect of FFA. The same mechanism (inhibition of utilization of FFA derived from endogenous triacylglycerols) should underlie at least in part the effects of etomoxir after its presence during culture. Thus etomoxir forms covalent bonds with the CPT I enzyme (19–21) causing irreversible inhibition of this enzyme. This leads to a prolonged effect of etomoxir which extends to the test period following culture. The failure of etomoxir to reverse inhibition by previous octanoate is probably due to the buildup of triacylglycerols containing residues of octanoic acid during exposure to octanoate. Subsequent lipolysis would then yield predominantly such residues which can be transported into mitochondria for β-oxidation also in the presence of etomoxir.

The present study demonstrates an influence of long-term FFA not only on glucose-induced insulin secretion but, to our knowledge for the first time, an effect also on glucose-induced proinsulin and total protein biosynthesis in islets. The results with etomoxir indicate the importance of fatty acid oxidation also on this modality of glucose regulation. The findings on biosynthesis are compatible with the observed decrease in islet insulin content that was universally observed after FFA. Such decrease could also partially be explained by the increased insulin secretion which occurred after FFA exposure but before measurements of islet insulin contents.

Our data on glucose oxidation indicate that the inhibitory effects of FFA on glucose-induced insulin secretion and biosynthesis are exerted at the level of glucose oxidation. In previous studies glucose oxidation has repeatedly been shown to be linked to the insulin-releasing potency of the hexose (24, 25). In our experiments a 48-h culture with palmitate or oleate significantly decreased oxidation of D-[U-^{14}C]glucose, and this inhibition was reversed by etomoxir. Evidence obtained from other tissues indicate that inhibition of glucose metabolism by FFA is mainly exerted on the pyruvate dehydrogenase (PDH) enzyme complex (5). The failure of previous FFA to affect the insulin response to α-ketoisocaproic acid is compatible with such notion since this nutrient is oxidized through the citric acid cycle without involvement of PDH. Also we find that production of $^{14}CO_2$ from D-[3,4-^{14}C]glucose, which reflects the decarboxylation of pyruvate due to PDH activity, is depressed after long-term exposure to FFA.

In addition to the inhibitory effects of glucose-induced insulin secretion, FFA also exerted stimulatory effects on secretion. In the short-term perspective, such effects are well documented in previous studies (2, 3, 22, 23). Such effects may be linked to mitochondrial oxidation and/or the breakdown in the cytoplasm of long chain acyl-CoA esters to second messengers such as diacylglycerol (8). The present study clearly shows that, in contrast to the inhibitory effects of FFA, a long induction time was not necessary for a stimulatory effect of previous FFA. However, the time of reversibility did not differ between the stimulatory and inhibitory effects. The similarity in reversibility could plausibly be due to increased endogenous triacylglycerols being present for an extended period of time after culture with FFA, such stores delivering the substrate necessary for the previously established influences of FFA.

It is interesting to note that variations in glucose concentra-

tions during culture with FFA produced quite different results on secretion when the concentration of glucose in final incubations was 27 as compared to 3.3 mM glucose. The inhibitory effect of FFA on the subsequent response to 27 mM glucose was thus similar regardless of whether the culture medium contained 3.3, 11, or 27 mM glucose. Conversely, the enhancement by previous FFA of secretion at basal glucose was obliterated by increasing the glucose concentration during culture from 11 to 27 mM. Of possible relevance to the latter effect are previous findings, showing (in short-term experiments) that a high glucose concentration decreases the oxidation of palmitate (1, 6, 26).

What is the relevance of our results for in vivo conditions? The type of FFA and concentrations used bear on this question. The fact that our results were achieved using those fatty acids (palmitate and oleate) which are most common in rat and man supports relevance for in vivo conditions. Concerning concentrations, those of palmitate or oleate were about 25% of FFA in rat plasma (9); however, the non-protein-bound fraction was probably elevated since serum proteins including albumin, were only present in the 10% concentration of the fetal calf serum. The possible impact of these differences between culture medium and plasma on FFA effects remains to be evaluated.

Other observations support the notion that the long-term effects of FFA that we observed in vitro are operative in the B cell during physiological and pathophysiological conditions. First, and most important, several of the present findings are similar to our results in lipid-infused rats (9). Also in the previous study, an induction period of more than 6 h was necessary to induce inhibition of glucose-induced insulin secretion. As in the present study, such inhibition was also specific for glucose-induced insulin secretion. Second, the effects of a high fat diet resemble our results. Such a diet in diabetic mice leads to a decrease of glucose-induced insulin secretion, biosynthesis, and oxidation (27). Also, in suckling neonate rats (receiving a high fat nutrition), inhibition of FFA oxidation was shown to enhance glucose-induced insulin secretion (28). Third, in the present study, the inhibitory effect of FFA was present also after co-culture with a high glucose concentration implying that inhibition would be operative also under diabetic conditions.

In summary, fatty acids time-dependently inhibit glucose-induced insulin secretion and biosynthesis. The specificity of this effect, its linkage to fatty acid oxidation indicate that a glucose-fatty acid cycle is operative in the pancreatic B cells. Elevated FFA in the starved and diabetic state potentially participate in the evolution or persistence of B cell insensitivity.

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References

1. Malaisse, W. J., L. Best, S. Kawazu, F. Malaisse-Lagae, and A. Sener. 1983. The stimulus-secretion coupling of glucose-induced insulin release: fuel metabolism in islets deprived of exogenous nutrients. *Arch. Biochem. Biophys.* 224:102–110.

2. Crespin, S. R., W. B. Greenough, and D. Steinberg. 1973. Stimulation of insulin secretion by long-chain free fatty acids. *J. Clin. Invest.* 53:1979–1984.

3. Malaisse, W. J., and F. Malaisse-Lagae. 1968. Stimulation of insulin secretion by non-carbohydrate metabolites. *J. Lab. Clin. Med.* 72:438–448.

4. Randle, P. J., P. B. Garland, C. N. Hales, and E. A. Newsholme. 1963. The glucose fatty acid cycle, its role in insulin sensitivity and the metabolic disturbances in diabetes mellitus. *Lancet* i:785–789.

5. Randle, P. J., A. L. Kerbey, and J. Espinal. 1988. Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. *Diabetes Metab. Rev.* 4:623–638.

6. Berne, C. 1975. The oxidation of fatty acids and ketone bodies in mouse pancreatic islets. *Biochem. J.* 152:661–666.

7. Malaisse, W. J., F. Malaisse-Lagae, A. Sener, and C. Hellerström. 1985. Participation of endogenous fatty acids in the secretory activity of the pancreatic B-cells. *Biochem. J.* 227:995–1002.

8. Prentki, M., S. Vischer, M. G. Glennon, R. Regazzi, J. T. Deeney, and B. E. Corkey. 1992. Malonyl-CoA and long chain Acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J. Biol. Chem.* 267:5802–5810.

9. Sako, Y., and V. Grill. 1990. A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B-cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology.* 127:1580–1589.

10. Ferrannini, E., E. J. Barrett, S. Bevilacqua, and R. A. De Fronzo. 1983. Effect of fatty acids on glucose production and utilization in man. *J. Clin. Invest.* 72:1737–1747.

11. MacGarry, J. D. 1992. What if Minkowski had been ageusic? an alternative angle on diabetes. *Science (Wash. DC).* 258:766–770.

12. Lacy, P. E., and M. Kostianovsky. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes.* 16:35–39.

13. Umbeit, W. W., R. H. Burris, and J. F. Stauffer. 1957. Manometric Techniques. Burgess Publishing Co., Minneapolis, MN. 149–150.

14. Halban, P. A., C. B. Wollheim, B. Blondel, and A. E. Renold. 1980. Long-term exposure of isolated pancreatic islets to mannoheptulose: evidence for insulin degradation in the cell. *Biochem. Pharmacol.* 29:2625–2633.

15. Keen, H., J. B. Field, and I. H. Pastan. 1963. A simple method for in vitro metabolic studies using small volumes of tissue and medium. *Metab. Clin. Exp.* 12:143–147.

16. Sener, A., and W. J. Malaisse. 1987. Stimulation by D-glucose of mitochondrial oxidative events in islet cells. *Biochem. J.* 246:89–95.

17. Herbert, V., K. S. Lau, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* 25:1375–1384.

18. Grill, V., M. Rundfeldt, and S. Efendic. 1981. Previous exposure to glucose enhances somatostatin secretion from the isolated perfused rat pancreas. *Diabetologia.* 30:495–500.

19. Wolf, H. P. O., K. Eistetter, and G. Ludwig. 1982. Phenylalkyloxirane carboxylic acids, a new class of hypoglycaemic substances: hypoglycaemic and hypoketonaemic effects of sodium 2-[5-(4-chlorophenyl)-pentyl-oxirane-2-carboxylate (B-807-27) in fasted animals. *Diabetologia.* 22:456–463.

20. Declercq, P. E., J. R. Falck, M. Kuwajima, H. Tyminski, D. W. Foster, and J. D. McGarry. 1987. Characterization of the mitochondrial carnitine palmitoyl-transferase enzyme system. Use of inhibitors. *J. Biol. Chem.* 262:9812–9821.

21. Foley, J. E. 1992. Rationale and application of fatty acid oxidation inhibitors in treatment of diabettes mellitus. *Diabetes Care.* 15:773–784.

22. Campillo, J. E., M. M. Valdivia, E. Rodriguez, and C. Osorio. 1979. Effect of oleic acid and octanoic acid on glucose-induced insulin release in vitro. *Diabète Metab.* 5:183–187.

23. Goberna, R., J. Jr Tamarit, R. Fussgänger, J. Tamarit, and E. F. Pfeiffer. 1974. Action of -hydroxybutyrate, acetoacetate and palmitate on the insulin release from the perfused isolated rat pancreas. *Horm. Metab. Res.* 6:256–260.

24. Malaisse, W. J. 1984. Insulin release: the fuel concept. *Diabètes Metab.* 9:313–320.

25. Meglasson, M. D., and F. M. Matschinsky. 1986. Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab. Rev.* 2:163–214.

26. Tamarit-Rodriguez, J., E. Vara, J. Tamarit. 1984. Starvation-induced changes of palmitate metabolism and insulin secretion in isolated rat islets stimulated by glucose. *Biochem. J.* 221:317–324.

27. Capito, K., S. E. Hansen, C. J. Hedeskov, and P. Thams. 1992. Fat-induced changes in mouse pancreatic islet insulin secretion, insulin biosynthesis and glucose metabolism. *Acta Diabetol.* 28:193–198.

28. Bliss, C. R., and G. W. G. Sharp. 1992. Glucose-induced insulin release in islets of young rat: time-dependent potentiation and effect of 2-bromostearate. *Am. J. Physiol.* 263(Endocrinol. Metab. 26):E890–E896.

EXHIBIT F

EXHIBIT G